

Haemodynamic, endocrine and renal actions of adrenomedullin 5 in an ovine model of heart failure

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A B S T R A C T

AM5 (adrenomedullin 5), a newly described member of the CGRP (calcitonin gene-related peptide) family, is reported to play a role in normal cardiovascular physiology. The effects of AM5 in HF (heart failure), however, have not been investigated. In the present study, we intravenously infused two incremental doses of AM5 (10 and 100 ng/min per kg of body weight each for 90 min) into eight sheep with pacing-induced HF. Compared with time-matched vehicle control infusions, AM5 produced progressive and dose-dependent increases in left ventricular $dP/dt(\max)$ [LD (low dose), + 56 mmHg/s and HD (high dose), + 152 mmHg/s] and cardiac output (+ 0.83 l/min and + 1.81 l/min), together with decrements in calculated total peripheral resistance (– 9.4 mmHg/min per litre and – 14.7 mmHg/min per litre), mean arterial pressure (– 2.8 mmHg and – 8.4 mmHg) and LAP (left atrial pressure; – 2.6 mmHg and – 5.6 mmHg) (all $P < 0.001$). HD AM5 significantly raised PRA (plasma renin activity) (3.5-fold increment, $P < 0.001$), whereas plasma aldosterone levels were unchanged over the intra-infusion period and actually fell in the post-infusion period (70% decrement, $P < 0.01$), resulting in a marked decrease in the aldosterone/PRA ratio ($P < 0.01$). Despite falls in LAP, plasma atrial natriuretic peptide and B-type natriuretic peptide concentrations were maintained relative to controls. AM5 infusion also induced significant increases in urine volume (HD 2-fold increment, $P < 0.05$) and urine sodium (2.7-fold increment, $P < 0.01$), potassium (1.7-fold increment, $P < 0.05$) and creatinine (1.4-fold increment, $P < 0.05$) excretion and creatinine clearance (60% increment, $P < 0.05$). In conclusion, AM5 has significant haemodynamic, endocrine and renal actions in experimental HF likely to be protective and compensatory in this setting. These results suggest that AM5 may have potential as a therapeutic agent in human HF.

INTRODUCTION

The AMs (adrenomedullins) are a group of regulatory peptides belonging to the CGRP (calcitonin gene-related peptide) superfamily, all of which share a conserved

six-amino-acid intramolecular ring structure formed by a single disulfide linkage and an amidated C-terminus [1]. Although five members of the AM subfamily have been identified in fish and named AM1–AM5 [2], until recently only the first two of these peptides were

Key words: adrenomedullin 5, haemodynamics, heart failure, hormone, renal function.

Abbreviations: AM5, adrenomedullin 5; AngII, angiotensin II; ANP, atrial natriuretic peptide; AVP, arginine vasopressin; BNP, B-type natriuretic peptide; BP, blood pressure; CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; CO, cardiac output; CTPR, calculated total peripheral resistance; HD, high dose; HF, heart failure; i.c.v., intracerebroventricular; i.v., intravenous; LAP, left atrial pressure; LD, low dose; LV, left ventricular; MAP, mean arterial pressure; NEP, neutral endopeptidase; PRA, plasma renin activity; RAMP, receptor activity-modifying protein.

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shown to have mammalian equivalents: AM [3] and AM2 [4] (also known as intermedin). In mammals, AM and AM2 are expressed throughout the central nervous system and in multiple peripheral organs including the heart, vasculature, kidneys and gastrointestinal tract [5,6]. While both peptides are well recognized as being multifunctional, their major role appears to be related to BP (blood pressure)/volume homeostasis, with actions that include direct vasodilation [7], positive inotropism [8,9] and diuresis and natriuresis [10]. This combination of haemodynamic and renal effects appears beneficial in studies of experimental and human HF (heart failure) [11–14], a state characterized by vasoconstriction and fluid/sodium retention. Accordingly, these peptides are viewed as potential therapeutic candidates in this disease.

In 2006, Ogoshi et al. [15] identified the existence of mammalian genes encoding AM5, and subsequent work by this group demonstrated the highly conserved nature of this peptide in mammals [16]. Bioactivity reported for AM5 to date includes potent osmoregulatory effects in fish (with a greater efficacy than AM1) [17], whereas intravenous administration in normal rats [16] and sheep [18] produces significant haemodynamic actions including decreases in peripheral resistance and arterial pressure together with increases in HR (heart rate) and CO (cardiac output) with a potency comparable with that seen with AM and AM2 [16,19,20]. Although these findings point towards a role for AM5 in the regulation of the cardiovascular system and body fluids in health, there have been no studies investigating the effects of AM5 in heart disease. Consequently, we investigated for the first time the integrated haemodynamic, endocrine and renal effects of AM5 in HF using an experimental ovine model.

MATERIALS AND METHODS

Surgical preparation

The study protocol was approved by the Animal Ethics Committee of the University of Otago, Christchurch. Eight Coopworth ewes (48–57 kg) were instrumented as described previously [21] via a left lateral thoracotomy under general anaesthesia [induced by i.v. (intravenous) thiopentone (15 mg/kg of body weight); maintained with 2.5% isoflurane, 2 l/min nitrous oxide and 2 l/min oxygen] and using approved peri-/post-operative analgesia [intercostal bupivacaine 0.5%/lignocaine 2%; i.v. carprofen (4 mg/kg of body weight); i.v. buprenorphine (0.005–0.01 mg/kg of body weight)]. Briefly, two polyvinyl chloride catheters were inserted in the left atrium for blood sampling and measurement of LAP (left atrial pressure); a Konigsberg pressure-tip transducer inserted in the aorta to record MAP (mean arterial pressure) and into the apex of the left ventricle to obtain maximum derivatives of pressure over time [LV (left ventricular) $dP/dt(\max)$] as an index

of contractility; an electromagnetic flow probe placed around the ascending aorta to measure CO; a Swan-Ganz catheter inserted in the pulmonary artery for administration of treatments; and a 7 French His-bundle electrode stitched subepicardially to the wall of the left ventricle for subsequent rapid 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, fed on a standard laboratory diet (500 g of sheep pellets and 250 g of chaff/day, containing 80 mmol sodium and 200 mmol potassium) and had free access to water.

Study protocol

HF was induced by 7 days of rapid LV pacing at 225 beats/min [21,22] and maintained by continuous pacing for the duration of the study. On days 8 and 10 of pacing, each sheep received vehicle control (0.9% saline) or porcine AM5 (Phoenix Pharmaceuticals) according to a balanced random order design. AM5 was infused at two incremental doses of 10 ng/min per kg of body weight for 90 min [LD (low dose)] followed immediately by 100 ng/min per kg of body weight for a further 90 min [HD (high dose)]. Both vehicle control and AM5 infusions were administered in a total volume of 43 ml (0.9% saline) via the pulmonary artery catheter commencing at 10:00 hours.

Using an online data acquisition system (PowerLab Systems; AD Instruments), haemodynamic recordings [MAP, LAP, CO, LV $dP/dt(\max)$ and CTPR (calculated total peripheral resistance; $CTPR = MAP/CO$)] were performed at 15 min intervals in the hour prior to treatment (baseline) and at 15, 30, 45, 60 and 90 min during both infusions and the post-infusion period. All measurements were carried out with the sheep standing quietly in the metabolic cage.

Blood samples were drawn from the left atrium 30 min and immediately pre-treatment (baseline) and at 30, 60 and 90 min during both infusions and the post-infusion period. Samples were taken into EDTA tubes on ice, centrifuged at 4°C and stored at either –20°C or –80°C before assay for the measurement of cAMP, PRA (plasma renin activity), aldosterone, AVP (arginine vasopressin), cortisol, endothelin-1, ANP (atrial natriuretic peptide), BNP (B-type natriuretic peptide), adrenaline and noradrenaline [23]. For each hormone, all samples from individual animals were measured in the same assay to avoid inter-assay variability. Haematocrit was measured with every blood sample taken. Samples for analysis of plasma sodium, potassium and creatinine concentrations were drawn into heparin tubes at 90 min intervals starting immediately pre-treatment.

Water intake and urine collections for the measurement of volume and sodium, potassium, creatinine and cAMP

excretion were made at 90 min intervals starting 90 min before treatment.

Statistics

Results are expressed as means \pm S.E.M. Baseline haemodynamic and hormonal values represent the mean of measurements made within the hour immediately pre-treatment. Differences between control and AM5 arms of the study (treatment \times time interactions) were analysed by two-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 was determined by Fisher's protected least-significant difference tests.

RESULTS

Similar to previous studies [21,22], rapid LV pacing at 225 beats/min for 7 days in the present study produced the haemodynamic, endocrine and sodium-retaining hallmarks of congestive HF, with reduced CO, MAP and renal function, elevated LAP and peripheral resistance and broad neurohormonal activation.

Compared with time-matched vehicle controls, infusion of AM5 produced progressive and dose-dependent increases in LV $dP/dt(\max)$ (LD +56 mmHg/s, HD +152 mmHg/s) and CO (LD +0.83 l/min, HD +1.81 l/min), together with substantial decrements in MAP (LD -2.8 mmHg, HD -8.4 mmHg), LAP (LD -2.6 mmHg, HD -5.6 mmHg) and CTPR (LD -9.4 mmHg/min per litre, HD -14.7 mmHg/min per litre; all $P < 0.001$; Figure 1). As seen in Figure 1, significant changes were observed during both the LD and HD infusions for all haemodynamic measurements. Following termination of AM5 administration, LV $dP/dt(\max)$, CO, LAP and CTPR gradually returned to control levels over the subsequent 30–90 min, whereas MAP rebounded above time-matched control values during the 45–90 min interval post-infusion. Haematocrit was significantly reduced by AM5 both during and after the infusion period ($P < 0.001$; Table 1).

PRA levels were unchanged during the LD AM5 infusion, but were significantly elevated in response to the HD (+1.77 nmol/h per litre; $P < 0.001$), with levels gradually returning to control concentrations over the 90 min post-infusion period (Figure 2). In contrast, plasma aldosterone levels were unaltered by either LD or HD AM5 administration, but decreased significantly and progressively on cessation of treatment ($P < 0.01$; Figure 2). These differences in responses were reflected in marked reductions in the aldosterone/PRA ratio over the HD and post-infusion periods ($P < 0.01$; Table 1). Non-dose-related trends for plasma cortisol to increase during AM5 infusion did not achieve statistical significance (Figure 2).

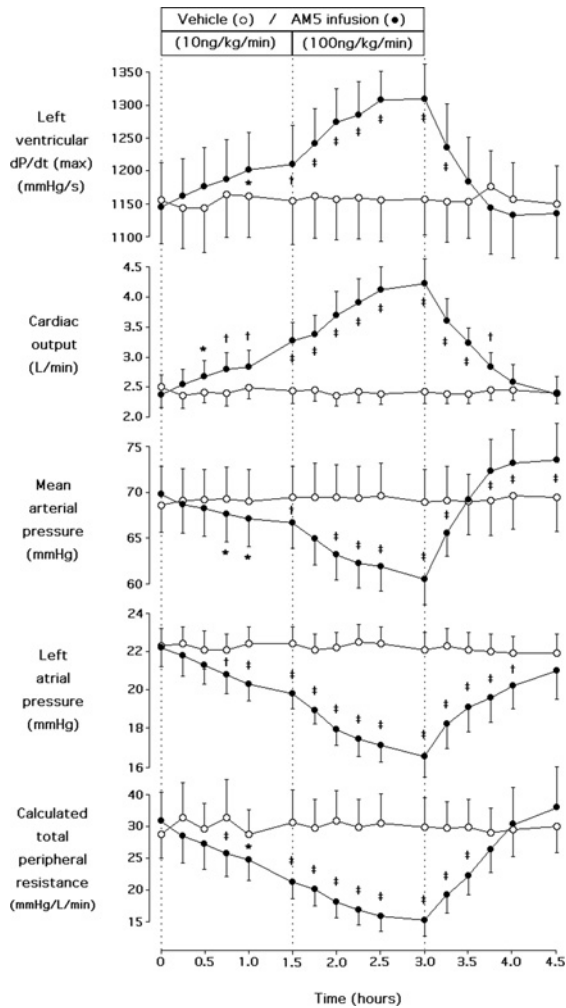


Figure 1 Haemodynamic responses to intravenous infusions of AM5 and vehicle control in eight sheep with HF

Values are means \pm S.E.M. Significant differences between time-matched control and AM5 data are shown by * $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$.

Plasma cAMP levels fell initially during LD AM5 and rose progressively during the HD infusion, before falling again below time-matched control values post-infusion ($P < 0.01$; Figure 3). Despite clear reductions in LAP, plasma ANP and BNP concentrations were maintained during AM5 infusions and showed a tendency to increase post-infusion (both not significant; Figure 3). Plasma potassium concentrations declined relative to controls over the HD and post-treatment periods (Table 1), while plasma noradrenaline, adrenaline, AVP, endothelin-1, sodium and creatinine levels were not significantly altered by AM5 administration (Table 1).

AM5 induced significant increases in urine output ($P < 0.05$) and urine sodium ($P < 0.01$), potassium and cAMP excretion (both $P < 0.05$) during the HD infusion, with increased output continuing during the post-infusion period (Figure 4). Creatinine excretion (Figure 4)

Table 1 Effects of AM5 in sheep with HF

Values are means \pm S.E.M. responses to consecutive 90 min intravenous infusions of vehicle control and AM5 at 10 ng/min per kg of body weight (LD) and 100 ng/min per kg of body weight (HD) in eight sheep with HF. Significant differences are shown by * $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$.

Variable	Baseline (0 min)	LD (90 min)	HD (180 min)	Post-infusion (270 min)
Haematocrit (%)				
Control	27.2 \pm 0.9	26.4 \pm 0.9	25.6 \pm 0.8	25.6 \pm 0.9
AM5	27.0 \pm 1.1	25.6 \pm 1.0†	23.7 \pm 1.0‡	22.4 \pm 1.0‡
Aldosterone/PRA ratio				
Control	1447 \pm 571	1609 \pm 496	1975 \pm 867	2446 \pm 1025
AM5	1324 \pm 405	1552 \pm 457	627 \pm 385‡	855 \pm 590‡
Plasma noradrenaline (pmol/l)				
Control	6294 \pm 1829	12824 \pm 3752	8754 \pm 1778	11987 \pm 3664
AM5	4273 \pm 787	8421 \pm 1389	9032 \pm 2640	9910 \pm 2111
Plasma adrenaline (epinephrine) (pmol/l)				
Control	401 \pm 73	584 \pm 171	475 \pm 106	482 \pm 89
AM5	338 \pm 56	497 \pm 92	484 \pm 113	403 \pm 76
Plasma vasopressin (pmol/l)				
Control	2.01 \pm 0.14	2.46 \pm 0.47	1.99 \pm 0.26	2.28 \pm 0.29
AM5	2.17 \pm 0.49	2.19 \pm 0.44	2.25 \pm 0.45	1.46 \pm 0.21
Plasma endothelin-1 (pmol/l)				
Control	4.89 \pm 0.73	5.27 \pm 0.80	5.42 \pm 0.98	5.03 \pm 0.77
AM5	5.20 \pm 0.80	5.33 \pm 0.70	4.86 \pm 0.68	5.29 \pm 0.70
Plasma sodium (mmol/l)				
Control	144.4 \pm 1.1	144.9 \pm 1.2	144.5 \pm 0.8	144.3 \pm 0.5
AM5	143.6 \pm 0.6	143.9 \pm 0.4	143.4 \pm 0.3	143.1 \pm 0.7
Plasma potassium (mmol/l)				
Control	4.25 \pm 0.19	4.33 \pm 0.19	4.35 \pm 0.19	4.36 \pm 0.20
AM5	4.09 \pm 0.12	4.23 \pm 0.14	4.04 \pm 0.15‡	3.71 \pm 0.11‡
Plasma creatinine (μmol/l)				
Control	81.3 \pm 2.3	80.3 \pm 2.3	79.5 \pm 1.6	81.1 \pm 2.9
AM5	84.9 \pm 3.8	85.3 \pm 3.6	82.3 \pm 3.3	81.8 \pm 3.9
Creatinine clearance (ml/min)				
Control	50.2 \pm 5.4	47.8 \pm 7.1	49.1 \pm 6.9	46.5 \pm 7.2
AM5	54.0 \pm 8.9	62.7 \pm 5.5*	76.3 \pm 7.9‡	56.2 \pm 11.7
Water intake (ml/1.5 h)				
Control	450 \pm 75	496 \pm 106	169 \pm 74	250 \pm 73
AM5	591 \pm 167	294 \pm 116	309 \pm 81	205 \pm 59

and creatinine clearance (Table 1; both $P < 0.05$) were augmented by both doses of AM5. Drinking was unchanged by AM5 (Table 1).

DISCUSSION

The present study investigated for the first time the integrated haemodynamic, endocrine and renal effects of AM5 administration in experimental HF. AM5 induced significant and dose-dependent reductions in peripheral resistance and arterial and atrial pressures, together with increases in cardiac contractility and output. Although AM5 activated PRA, plasma aldosterone levels were suppressed and natriuretic peptide concentrations

were maintained despite the decline in LAP. AM5 also enhanced renal function, producing significant increases in urine volume, sodium excretion and creatinine clearance.

Members of the CGRP family are believed to exert their biological effects in mammals principally through interactions with combinations of the G-protein-coupled CLR (calcitonin receptor-like receptor) and three RAMPs (receptor activity-modifying proteins), resulting in the generation of the intracellular second messenger, cAMP [24]. Although plasma cAMP concentrations tended to increase during the HD AM5 infusion in the present study, levels did not rise significantly above control, and the LD infusion period was characterized by clear-cut reductions in cAMP. This response differs

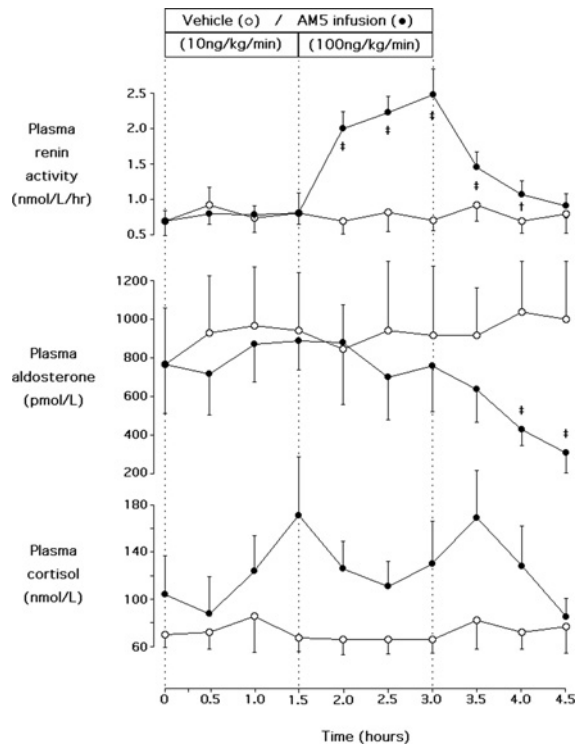


Figure 2 PRA, aldosterone and cortisol responses to intravenous infusions of AM5 and vehicle control in eight sheep with HF

Values are means \pm S.E.M. Significant differences between time-matched control and AM5 data are shown by $\dagger P < 0.01$ and $\ddagger P < 0.001$.

somewhat from that observed previously with identical doses of AM [11] and AM2 [12] in sheep with HF, where plasma cAMP levels were maintained during the LD infusion and significantly increased compared with the control during the HD (Table 2). Our findings with AM5 are consistent with the results from a study by Takei et al. [16] that found the potency of AM5 for cAMP accumulation (in COS-7 cells) with any CLR-RAMP combination was much lower than AM, although the cardiovascular effects of the two peptides [i.v. and i.c.v. (intracerebroventricular) in rats] were comparable. This observation led the authors to surmise that AM5 may have as yet unidentified specific receptor(s) [and second messenger(s)] that differ from CLR-RAMP complexes. However, the decrease in circulating cAMP concentrations seen during LD AM5 in the present study probably reflects the haemodynamic improvement noted during this period [25]. Despite continued haemodynamic improvement during HD AM5, cAMP levels rose, suggesting that AM5 did activate this second messenger and may indeed signal via the same receptors as AM and AM2. Of note, AM5 administration in normal sheep was associated with significant increases in circulating cAMP [18], albeit by amounts less than half

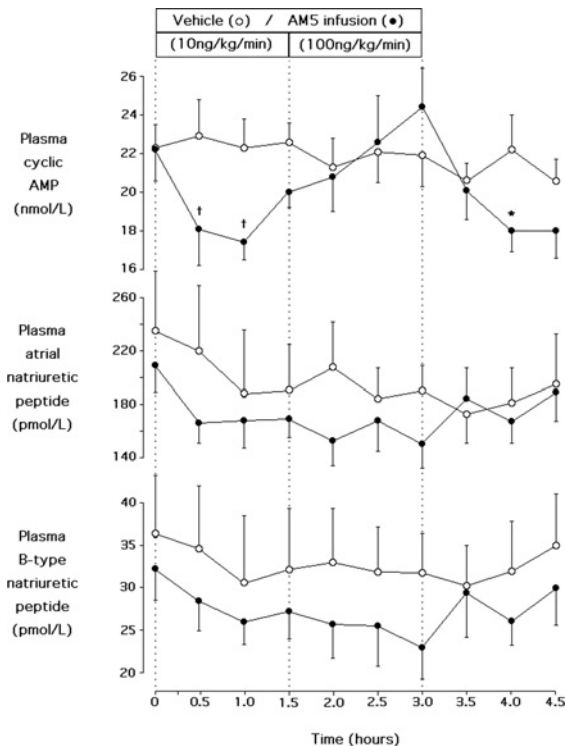


Figure 3 Plasma cAMP, ANP and BNP responses to intravenous infusions of AM5 and vehicle control in eight sheep with HF

Values are means \pm S.E.M. Significant differences between time-matched control and AM2 data are shown by $*P < 0.05$ and $\dagger P < 0.01$.

that seen with equivalent doses of AM [19] and AM2 [20] (Table 2).

Despite a possible reduced plasma cAMP response to AM5 relative to AM and AM2, we found that the haemodynamic effects of AM5 were largely comparable in terms of magnitude and temporal profile to that produced by the other two AM peptides in this setting of experimental HF [11,12] (Table 2). Similar comparative potencies for all three AM peptides have previously been reported in normal rats [16] and sheep [19] (Table 2). Given these similarities, it is likely that the mechanisms underlying these effects are analogous for the three peptides. The hypotensive actions of AM and AM2 are reported to be due to direct vasodilatory actions both receptor mediated and via stimulation of nitric oxide [7], and the concurrent falls in MAP and peripheral resistance seen with AM5 in the present study also suggest a direct affect of this peptide on arterial tone. A similar correlation between falls in BP and CTPR was noted in normal sheep with AM5 administration [18] (Table 2). In comparison with AM, where reductions in MAP persisted for 1 h post-infusion [11], MAP responses were more transient with AM5 (the present study) and AM2 [12] (absent after 30 min for both peptides). Takei

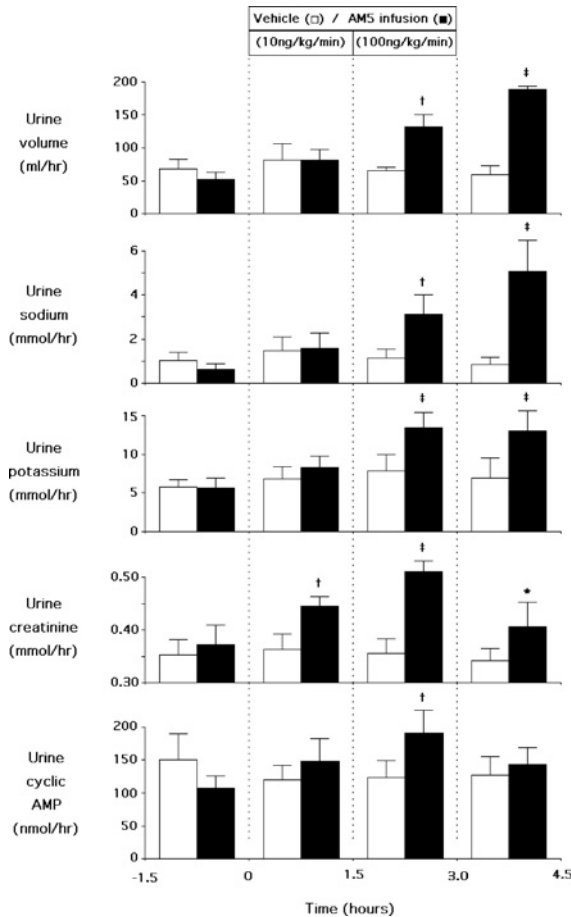


Figure 4 Renal responses to intravenous infusions of AM5 and vehicle control in eight sheep with HF

Values are means \pm S.E.M. Significant differences between time-matched control and AM2 data are shown by * $P < 0.05$, † $P < 0.01$ and ‡ $P < 0.001$.

et al. [16] also observed a more short-lived BP effect of AM5 relative to AM following i.c.v. administration of the peptides in rats. This difference in the time course suggests that the vasodilatory actions of AM5, like AM2, may be mediated by CLR–RAMP complexes and/or other unidentified receptors different from those mediating the AM effect [26]. Clearly, additional studies are needed to clarify this issue.

As observed previously in normal sheep [18], AM5 induced significant and dose-dependent augmentation of CO in the setting of HF. Although this effect is probably attributable in part to the attendant reduction in cardiac afterload (evidenced by falls in CTPR and MAP), it is plausible that AM5 also exhibits positive inotropic activity as the rise in CO was associated with prominent increases in LV $dP/dt(\max)$. Similar increases in CO and $dP/dt(\max)$ have been observed with AM and AM2 administration (Table 2) (in conjunction with enhanced coronary artery perfusion flow) [11,12,27], and both peptides are reported to have direct inotropic actions in

isolated perfused rat hearts [8,9]. The AM5-induced rise in CO in the present study presumably contributed to the fall in LAP, although it is also possible that AM5 possesses venodilator actions as has been demonstrated for AM2 [28]. A decrease in blood volume cannot be implicated as the significant decline in LAP seen during the LD AM5 infusion occurred well before the increase in urine output and in the face of a fall in haematocrit. A reduction in haematocrit has been recorded previously following AM5 administration in normal sheep [18] as well as during AM2 infusion in HF animals [12], and suggests a shift of fluid from the extravascular to intravascular compartment. The mechanisms underlying this effect are yet to be determined for any of the AM peptides.

AM5 induced a significant rise in PRA during the HD infusion period in HF sheep, an effect also noted for the peptide in normal animals [18] and similar to that seen with AM and AM2 in experimental HF [11,12] (Table 2). Although the activation of renin is likely to be due, at least in part, to the decline in arterial pressure seen during this period (via stimulation of afferent renal arteriolar baroreceptors), it is also feasible that AM5 may directly enhance renin release from the juxtaglomerular cells in the kidney as previously demonstrated for AM [29]. Although AM5 increased PRA [and presumably AngII (angiotensin II)] levels in the present study, there was no corresponding rise in plasma aldosterone. Indeed, aldosterone concentrations were unaltered during HD AM5 and significantly reduced post-infusion (at which time PRA was returning to control levels), resulting in a considerable decline in the aldosterone/PRA ratio and indicating inhibition of AngII-induced aldosterone secretion. It is probable that AM5 has direct inhibitory actions at the adrenal glomerulosa similar to those demonstrated for the AM peptide [30] given the reports of AM5 gene expression in the adrenal gland [16]. Contrary to our results in sheep with HF, AM5 induced a rise in aldosterone in normal sheep [18] (Table 2). However, this increment was minor in relation to the concomitant increase in PRA with a resultant decrease in the aldosterone/PRA ratio similar to that seen in the present study in the setting of HF. The declines in plasma potassium concentrations observed with AM5 administration in HF sheep may also have contributed to the reduction in aldosterone.

Plasma concentrations of ANP and BNP were maintained relative to control during AM5 infusion in the present study despite clear falls in LAP and arterial pressure (indicating reduced stimulus for secretion and release of these peptides). Similar ANP/BNP responses have been observed with AM and AM2 administration in HF sheep [11,12], and suggest that the AM peptides can either enhance secretion of ANP/BNP or alter their clearance from the circulation to account for the discrepancy between their sustained plasma levels and the pronounced falls in LAP and MAP during the

Table 2 Comparative effects of the AM peptides in normal and HF sheep

Summary and comparison of the major biological effects of AM, AM2 and AM5 in normal and HF sheep using an identical study design. Arrows indicate the maximum responses to each peptide during the HD infusion period (100 ng/min per kg of body weight over 90 min) indicated by arrows (minor, moderate or large). ND, no data; —, no significant change; right atrial pressure measured in normal animals, left atrial pressure measured in HF.

Variable	Data source	AM	AM2	AM5
	Normal HF	Charles et al. [19] Rademaker et al. [11]	Charles et al. [20] Rademaker et al. [12]	Charles et al. [18] The present study
HR (beats/min)				
Normal		↑↑	↑↑↑	↑↑↑
HF		ND	ND	ND
CO (l/min)				
Normal		↑↑↑	↑↑↑	↑↑↑
HF		↑	↑↑	↑↑
dP/dt(max) (mmHg/s)				
Normal		ND	ND	ND
HF		ND	↑↑↑	↑↑↑
MAP (mmHg)				
Normal		↓↓↓	↓↓↓	↓↓↓
HF		↓↓	↓↓	↓↓
Peripheral resistance (mmHg/min per litre)				
Normal		↓↓	↓↓	↓↓
HF		↓↓↓	↓↓↓	↓↓↓
Right/left atrial pressure (mmHg)				
Normal		↓	—	—
HF		↓↓	↓↓	↓↓
Plasma cAMP (pmol/l)				
Normal		↑↑	↑↑↑	↑
HF		↑	↑↑	—
PRA (nmol/l per h)				
Normal		↑↑	↑↑↑	↑
HF		↑	↑↑	↑↑
Plasma aldosterone (pmol/l)				
Normal		↑	↑↑	↑
HF		↓	—	—
Urine volume (ml/h)				
Normal		—	—	—
HF		↑↑	↑↑	↑↑
Urine sodium excretion (mmol/h)				
Normal		—	—	—
HF		↑↑↑	↑↑↑	↑↑↑

infusion period. While a possible direct stimulatory effect of AM5 on natriuretic peptide secretion is yet to be investigated, it is conceivable that AM5 modifies some other regulatory mechanism of natriuretic peptide production, such as AngII (indicated here by increased PRA levels), which has been shown to directly stimulate natriuretic peptide secretion from the heart [31]. Alternatively, as has been shown for AM, AM5 may enhance the natriuretic peptide secretory responses to AngII [32], endothelin-1 [33] and/or volume loading [34], all of which are activated in, or characteristic of, the HF state. Alternatively, AM5 may alter the

clearance of ANP/BNP. Although no information is as yet available on the newly identified mammalian AM5 peptide, it appears likely that AM is degraded initially by metalloproteases [35] and several *in vivo* studies provide (indirect) evidence that NEP (neutral endopeptidase), an enzyme significantly involved in the clearance of the natriuretic peptides [36], also contributes to the degradation of AM, with plasma AM levels seen to increase following NEP inhibition [37,38]. Thus possible competition for the enzyme during AM5 administration might result in the preservation of plasma ANP/BNP concentrations observed in the present study. However,

in vitro work suggests that NEP is not significantly involved in the metabolism of AM [35]. Clearly, further investigations are required, but whatever the mechanisms, the enhancement of natriuretic peptide levels in HF by AM5 is favourable [36].

AM5 administration at the higher dose produced significant increments in urine output and urine sodium and creatinine excretion in sheep with HF. Impressively, these effects occurred in the face of substantial falls in arterial pressure (and therefore presumably renal perfusion pressure). These data contrast with findings in normal sheep [18] and rats [16] where no significant renal effects of AM5 were observed. However, it should be noted that in these studies in normal animals, urine volume and sodium were maintained despite the reductions in BP, suggesting a relative improvement in renal function in normal health also. Although the mechanisms underlying these renal actions of AM5 are yet to be established, it is possible that an increase in glomerular filtration may have contributed in light of the accompanying rise in creatinine clearance, as well as the observed decrease in circulating aldosterone levels. A similar enhancement of renal function has been demonstrated previously following identical doses of AM and AM2 in experimental ovine HF [11,12] (Table 2), with modes of action reported to include reductions in renal vascular resistance and increases in renal blood flow (via pre- and post-glomerular arteriolar vasodilation) and glomerular filtration rate, together with inhibition of proximal and distal fractional sodium reabsorption [37,39,40]. Similar direct actions of AM5 in the kidney might also be a possibility given the significant increase in urine cAMP levels.

In summary, the present study is the first to report the effects of AM5 in HF using an experimental ovine model and demonstrates that the peptide induces significant reductions in CTPR, MAP and LAP, together with improvements in cardiac contractility and output. AM5 activates PRA, but suppresses plasma aldosterone, maintains natriuretic peptide concentrations despite falls in LAP and enhances renal function. Our findings suggest that AM5, like AM and AM2, contributes to BP and volume homeostasis in HF. The spectrum of bioactivity exhibited by AM5 in experimental HF which, at least in the short term, are generally of a beneficial nature, raise the possibility that the peptide may have potential as a therapeutic agent in human HF.

AUTHOR CONTRIBUTION

Miriam Rademaker was responsible for the conceptualization and design of the research and for all 'hands on' aspects, including surgical instrumentation of animals, protocol execution, statistical analysis and interpretation of data, and graphics and paper generation. Christopher

Charles, Gary Nicholls and Mark Richards contributed to the study concept and design and the paper revision.

ACKNOWLEDGEMENTS

We are grateful to the Animal Laboratory staff of the University of Otago, Christchurch, for assistance with animal studies and Christchurch Cardioendocrine Laboratory staff for hormone assays.

FUNDING

This work was supported by the National Heart Foundation of New Zealand and the Health Research Council of New Zealand.

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Received 16 September 2011/26 October 2011; accepted 17 November 2011
 Published as Immediate Publication 17 November 2011, doi:10.1042/CS20110483