Long-Term Adrenomedullin Administration in Experimental Heart Failure

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Abstract—Short-term administration of adrenomedullin, a recently discovered peptide with potent vasodilator, natriuretic, and aldosterone-inhibitory actions, has beneficial effects in experimental and clinical heart failure. The effects of prolonged adrenomedullin administration have not previously been assessed in this setting. Consequently, in 16 sheep with pacing-induced heart failure, we infused either adrenomedullin (10 ng/kg per minute; n=8) or a vehicle control (Hemaccel; n=8) for 4 days. Compared with control data, infusion of adrenomedullin persistently increased circulating levels of the peptide (by 9.5 pmol/L; P<0.001), in association with prompt (15 minutes) and sustained (4 days) increases in cardiac output (day 4, 27%), and reductions in peripheral resistance (30%), mean arterial pressure (13%), and left atrial pressure (24%; all, P<0.001). Adrenomedullin also significantly increased urinary sodium excretion (day 4, 3-fold; P<0.05), creatinine excretion (1.2-fold; P<0.001), and creatinine clearance (1.4-fold; P<0.001) over the 4 days of treatment, whereas urine volume and cAMP excretion tended to be elevated (both, 0.1>P>0.05). Plasma renin activity was increased (P<0.05), whereas aldosterone levels were reduced in a sustained fashion (P<0.01). Plasma endothelin rose transiently (hours 1 to 6) after initiation of treatment (P<0.05). Despite substantial cardiac unloading, plasma concentrations of the natriuretic peptides were not significantly different from control. In conclusion, long-term administration of adrenomedullin induces pronounced and sustained cardiovascular and renal effects in experimental heart failure, including reductions in cardiac preload and afterload, as well as augmentation of cardiac output, sodium excretion, and glomerular filtration. These findings support the concept of adrenomedullin as a protective hormone during hemodynamic compromise with therapeutic potential in heart failure. (Hypertension. 2002;40:667-672.)

Key Words: adrenomedullin – heart failure – vasodilation – natriuresis – aldosterone

Adrenomedullin (AM) is a novel endogenous peptide that appears to be intimately involved in cardiovascular and pressure/volume homeostasis. AM is detected in a wide variety of tissues, and measurable levels circulate in normal human plasma (typically in the lower picomolar range). In heart failure (HF), cardiac production and secretion of AM are increased, and plasma levels of the peptide are elevated in proportion to the severity of cardiac and hemodynamic impairment. In addition, circulating levels of AM in this disease have been shown to provide independent prognostic information. These findings, together with the demonstrated biological effects of this peptide (including vasodilation and natriuresis), suggest AM may participate in a compensatory fashion in the pathophysiology of HF.

Recent reports have demonstrated that AM has beneficial actions in HF. These studies, performed in both experimental and human HF, have consistently shown that short-term (hours) administration of this peptide reduces arterial pressure and cardiac filling pressures and improves cardiac output (CO), in association with inhibition of plasma aldosterone (despite increased renin release) and augmentation of renal glomerular filtration and sodium excretion. These results suggest that AM may be an alternative or adjunctive strategy to vasodilating/volume eliminating agents in the treatment of this disease. However, the therapeutic potential of AM in HF cannot be fully determined until the effects of prolonged administration of the peptide have been evaluated. The present study examines for the first time the hemodynamic, hormonal, and renal effects of long-term (days) administration of AM in experimental HF.

Methods

Surgical Preparation

Sixteen Coopworth ewes (35 to 51 kg; Lincoln University Research Farm, Lincoln, Canterbury, New Zealand) were instrumented via a left lateral thoracotomy as previously described. Briefly, under general anesthesia (17 mg/kg thiopentone, halothane/nitrous oxide) 2 PVC catheters were inserted in the left atrium for blood sampling and left atrial pressure (LAP) determination; a Konigsberg pressure-tip transducer was inserted in the aorta to record mean arterial pressure (MAP); an electromagnetic flow probe was placed around the ascending aorta to measure CO; a Swan-Ganz catheter was inserted in the pulmonary artery for infusions, and an His-bundle electrode was stitched subepicardially to the wall of the left ventricle for left ventricular pacing. A bladder catheter was inserted per urethra for urine collections. Animals recovered for 14 days before starting the...
study protocol. During the study, the animals were held in metabolic cages, had free access to water, and ate a diet of chaff and sheep pellets (containing 80 mmol/day sodium and 200 mmol/day potassium).

**Study Protocol**

A state of HF was induced by 7 days of rapid left ventricular pacing at 225 bpm. With the pacing rate maintained, the sheep received a continuous 4-day intravenous infusion of either a vehicle control (Hemaccel, Hoechst Marion Roussel; n=8) or human AM (10 ng/kg per minute, synthesized as previously described; n=8). Infusions commenced at 1000 hours on study day 0 and were administered in a total volume of 50 mL/d. MAP, LAP, CO, and calculated total peripheral resistance (CTPR=MAP/CO) were recorded at 15-minute intervals in the hour before infusion on study day 0 (baseline), at 0.5, 1, 1.5, 2, 4, and 6 hours after commencement of treatment, and then daily (1000 hours) on study days 1 to 4. Hemodynamic measurements were determined by on-line computer-assisted analysis by use of established methods.

Blood samples were drawn from the left atrium (immediately after hemodynamic measurements) into tubes on ice, centrifuged at 4°C, and stored at −80°C before assay for AM, cAMP, atrial and brain natriuretic peptide (ANP and BNP, respectively), plasma renin activity (PRA), aldosterone, endothelin-1, catecholamines, and cortisol. All samples from individual animals were measured in the same assay to avoid interassay variability. Hematocrit and plasma sodium, potassium, creatinine, calcium, and glucose concentrations were measured in every blood sample taken. Urine volume and samples for the measurement of urine cAMP, sodium, potassium, and creatinine excretion were collected over the 2 hours before infusion on study day 0 (baseline); at 2, 4, and 6 hours after commencement of treatment; and then daily (1000 hours) on study days 1 to 4. Water intake was measured daily. The study protocol was approved by the local Animal Ethics Committee.

**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 infusion. Statistical analysis was performed by repeated-measures ANOVA. Statistical significance was assumed when P<0.05.

**Results**

Seven days of rapid left ventricular pacing induced the hemodynamic and hormonal hallmarks of established HF as observed in previous studies. Compared with normal values, for our laboratory, recorded in sheep before pacing (n=20), 7 days of rapid pacing in the present study resulted in significant reductions in MAP (normal data, 90±2 mm Hg; paced AM baseline data, 69±2 mm Hg; P<0.001) and CO (normal, 3.4±0.2 L/min; paced, 1.76±0.19 L/min; P<0.001) and increases in LAP (normal, 2.3±0.3 mm Hg; paced, 24.2±0.7 mm Hg; P<0.001) and plasma levels of ANP (normal, 12±1 pmol/L; paced, 229±34 pmol/L; P<0.001), BNP (normal 3.8±0.4 pmol/L; paced 56±7 pmol/L, P<0.001), PRA (normal, 0.39±0.06 nmol/L per hour; paced, 1.28±0.30 nmol/L per hour; P<0.001), aldosterone (normal, 362±41 pmol/L; paced, 1449±351 pmol/L; P<0.001), and endothelin-1 (normal, 1.9±0.1 pmol/L; paced, 4.1±0.6 pmol/L; P<0.01).

Compared with control data, infusion of AM induced prompt (15 minutes) and sustained (4 days) increases in CO (day 4, 27%), and reductions in LAP (24%), and CTPR (30%) (all, P<0.001) (Figure 1). Hematocrit was not significantly altered relative to control values (Table). Long-term AM administration significantly increased urinary sodium excretion (day 4, 3-fold; P<0.05), creatinine excretion (1.2 fold; P<0.01) (Figure 2), and creatinine clearance (1.4 fold; P<0.01) (Table) over the 4 days of treatment and tended to enhance urine volume and urine cAMP excretion (both, 0.1>P>0.05) (Figure 2). Plasma creatinine concentrations fell progressively during the AM infusion period (P<0.05) (Table), whereas urinary potassium excretion (Figure 2) and water intake (Table) were unchanged.

Infusion of AM increased circulating concentrations of the peptide—with levels plateauing at ~10.5 pmol/L by day 2 (P<0.001) (Figure 3). Plasma cAMP and natriuretic peptide levels did not change significantly over the 4-day AM infusion (Figure 3). PRA was elevated compared with control (P<0.05), whereas aldosterone concentrations were reduced (P<0.01) (Figure 4). Although plasma endothelin increased acutely (30 minutes to 6 hours) after initiation of the AM infusion, levels were not significantly different from control on days 1 to 4 (Figure 4). Circulating cortisol levels tended to be raised relative to control (P=0.0574) (Figure 4), whereas plasma norepinephrine, epinephrine, sodium, potassium, calcium, and glucose (Table) were not altered.
The peptide in both experimental and human HF. The previously in response to short-term (hours) administration of sheep with HF are qualitatively similar to those reported peptide, norepinephrine, and epinephrine levels were not aldosterone levels (despite a rise in PRA), whereas natriuretic increases in CO and reductions in CTPR, MAP, and LAP.

Sodium excretion, creatinine excretion, and creatinine clearance were significantly enhanced over the 4 days of treatment, and urine volume and cAMP excretion tended to be significantly altered.

The hemodynamic effects of prolonged infusion of AM in sheep with HF are qualitatively similar to those reported previously in response to short-term (hours) administration of the peptide in both experimental and human HF. The hypotensive action of AM in the present study, of rapid onset and persistent, is likely to be primarily owing to a direct affect on arterial tone, as reflected in concomitant reductions in CTPR. In addition, inhibitory effects of AM on the procontractile activities of factors such as angiotensin II and endothelin-1 may have played a contributory role in this setting, in which these systems are activated. AM appears to have lowered blood pressure without plasma volume contraction because there was no significant increase in either urine volume or hematocrit (or reduction in volume intake) during the 4-day infusion period. Our findings concur with studies in both normal and hypertensive rats in which 14-day infusions of AM resulted in persistent reductions in systolic blood pressure. In addition, transgenic mice overexpressing AM in their vasculature exhibit significantly lower blood pressure than that of their wild-type litter mates. The prompt and protracted augmentation of CO observed during the

**Discussion**

Long-term administration of AM induced pronounced and prolonged cardiovascular, endocrine, and renal effects in experimental HF. Long-term augmentation of plasma AM concentrations was associated with significant and sustained increases in CO and reductions in CTPR, MAP, and LAP. Sodium excretion, creatinine excretion, and creatinine clearance were significantly enhanced over the 4 days of treatment, and urine volume and cAMP excretion tended to be elevated. AM also induced sustained reductions in plasma aldosterone levels (despite a rise in PRA), whereas natriuretic peptide, norepinephrine, and epinephrine levels were not significantly altered.

The hemodynamic effects of prolonged infusion of AM in sheep with HF are qualitatively similar to those reported previously in response to short-term (hours) administration of the peptide in both experimental and human HF. The hypotensive action of AM in the present study, of rapid onset and persistent, is likely to be primarily owing to a direct affect on arterial tone, as reflected in concomitant reductions in CTPR. In addition, inhibitory effects of AM on the procontractile activities of factors such as angiotensin II and endothelin-1 may have played a contributory role in this setting, in which these systems are activated. AM appears to have lowered blood pressure without plasma volume contraction because there was no significant increase in either urine volume or hematocrit (or reduction in volume intake) during the 4-day infusion period. Our findings concur with studies in both normal and hypertensive rats in which 14-day infusions of AM resulted in persistent reductions in systolic blood pressure. In addition, transgenic mice overexpressing AM in their vasculature exhibit significantly lower blood pressure than that of their wild-type litter mates. The prompt and protracted augmentation of CO observed during the
present study, likely to be owing, at least in part, to the fall in left ventricular afterload, may also have been mediated via direct positive inotropic actions of AM. 20 These results suggest that AM may participate in both short- and long-term regulation of hemodynamic function.

The renal response to long-term AM administration in experimental HF included a sustained natriuresis and rise in creatinine clearance, as well a tendency for urine output and cAMP excretion to be elevated. A similar range of responses have previously been observed in animals and humans with HF 10 after short-term AM administration, and are likely to be owing to the actions of AM to directly reduce tubular sodium reabsorption and renal vascular resistance and to increase renal blood flow. 21–23 A persistent increase in glomerular filtration rate, as assessed by the rise in creatinine clearance, also appears to have contributed to the sustained natriuretic effect observed in the current study. The maintenance of sodium excretion in association with the protracted reduction in MAP (and presumably renal perfusion pressure) points to a shift in the pressure-natriuresis curve with prolonged AM administration. Conceivably, maintained circulating levels of the natriuretic peptides, despite falls in MAP and intracardiac pressure, contribute to this shift. Long-lasting renal effects in response to long-term augmentation of AM have formerly been demonstrated in normal and spontaneously hypertensive rats in which both urine and sodium excretion were maintained despite significant and protracted falls in blood pressure after low-dose infusion of AM (200 ng/hour) for 14 days. In another study in deoxycorticosterone acetate–salt hypertensive rats, tail-vein AM gene delivery enhanced renal function when evaluated 16 days after delivery, with a 3-fold increase in renal blood flow and 2-fold increase in glomerular filtration rate in association with significant increases in urinary cAMP excretion. 24 The ability of AM to enhance sodium excretion long-term in the face of sustained reductions in renal perfusion pressure in these sheep with HF, which are already in an underperfused state, could prove to be a major advantage in the clinical setting of cardiac failure.

Plasma levels of AM achieved over the 4-day infusion period (plateauing by day 2 at ~10.9 pmol/L) are well within the pathophysiological range observed in patients with HF.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Mean ± SEM urine volume, sodium, potassium, creatinine, and cAMP excretion responses to 4-day infusions of vehicle (open columns; n = 8) and adrenomedullin (filled columns; 10 ng/kg per minute; n = 8) in sheep with congestive HF. The baseline sample was collected over the 2 hours before treatment. Significant differences between vehicle and AM are shown by the following: *P < 0.05, **P < 0.01, and †P < 0.001.

![Figure 3](http://hyper.ahajournals.org/)

**Figure 3.** Mean ± SEM plasma AM, plasma cAMP, ANP, and BNP responses to 4-day infusions of vehicle (○; n = 8) and AM (●; 10 ng/kg per minute; n = 8) in sheep with congestive HF. The baseline data point represents the mean of 2 samples taken in the hour before treatment. Significant differences between vehicle and AM are shown by the following: *P < 0.01 and †P < 0.001.
Figure 4. Mean±SEM PRA, aldosterone, cortisol, and endothelin response responses to 4-day infusions of vehicle (○; n=8) and AM (●; 10 ng/kg per minute; n=8) in sheep with congestive HF. The baseline data point represents the mean of 2 samples taken in the hour before treatment. Significant differences between vehicle and AM are shown by the following: * P<0.05, ** P<0.01, and † P<0.001.

Even with this small increase in circulating levels, significant hemodynamic and renal effects were evident, suggesting that the raised levels of the peptide observed in this disease may play a protective compensatory role. The long-term elevation of plasma AM was not associated with significant rises in plasma levels of cAMP, a proposed second messenger of the peptide. The disassociation between increases in plasma AM and cAMP has been noted previously during short-term AM infusions in sheep with HF using an identical dose,8 with elevations in cAMP only evident at higher doses of AM. However, it is possible that cAMP was raised sufficiently at the tissue level in the present study to produce the significant responses that were observed. Alternatively, other signaling pathways were involved.1

Similar to studies using acute administration of AM,8 plasma levels of the natriuretic peptides were unaltered compared with control data during the first 6 hours of the AM infusion period, despite significant falls in LAP and therefore the stimulus for secretion. These findings contrast with previous investigations in which short-term administration of vasodilator agents such as ACE inhibitors demonstrate a close parallelism between falls in atrial pressure and plasma natriuretic peptides levels.23 In view of a report that AM may actually suppress ANP mRNA expression,26 it is possible that natriuretic peptide secretion was enhanced during this time by stimulatory factors such as endothelin-1,27 which was raised in a parallel fashion to that of ANP and BNP. Furthermore, a recent report by Horio et al28 has demonstrated that AM augments endothelin-stimulated ANP secretion from myocytes. From day 1 through to day 4, however, plasma natriuretic peptide concentrations (and endothelin-1 levels) tended to be reduced compared with control data—presumably as a consequence of the sustained improvements in hemodynamic function. The transient increase in plasma endothelin-1 levels may conceivably relate to stimulation by factors such as cortisol,29 which shows a concomitant tendency to rise in the present study, or to a reduction in shear stress and/or tissue oxygen concentrations after the AM-induced fall in blood pressure (from already reduced levels)—both of which are reported to enhance endothelin secretion.30,31 The acute rise in endothelin is unlikely to be owing to a direct effect of AM, given that the peptide has been shown to inhibit endothelin production in vascular smooth muscle cells.32 Indeed, increasing evidence indicates the existence of a paracrine/autocrine regulatory loop between AM and endothelin, with recent findings demonstrating that stimulation of the endothelin type B receptor induces the synthesis and secretion of AM in canine aortic endothelial cells,33 suggesting the peptide may function to buffer the vasoconstrictor effect of endothelin.

PRA was elevated significantly on days 3 and 4 of the present study. A predilection for AM to increase circulating levels of renin has been demonstrated previously during acute investigations of AM in HF,9 and may be secondary to activation of the afferent renal arteriolar baroreceptor (owing to the decline in renal perfusion pressure) or to direct stimulation by AM of the juxtaglomerular cells.34 Despite the rise in PRA, plasma aldosterone concentrations were reduced from days 1 to 4. These data concur with findings in normal and hypertensive rats after a 14-day infusion of AM,17 and is consistent with the known aldosterone-inhibitory actions of this peptide.35 The persistent reductions in plasma aldosterone may also have contributed to the sustained natriuresis observed in the present study. The acute rise in aldosterone observed in the first hour after initiation of AM treatment is likely to be in response to an increase in its secretagogue adrenocorticotropic hormone, as assessed by the concurrent rise in plasma cortisol levels.

In conclusion, we have demonstrated that long-term administration of AM induces pronounced and sustained cardiovascular, endocrine, and renal effects in experimental HF, including reductions in cardiac preload and afterload, and augmentation of CO, sodium excretion, and glomerular filtration together with suppression of aldosterone.

Perspectives

The findings of the present study further support the concept of AM as a protective hormone during hemodynamic compromise with therapeutic potential in HF.
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