Combined Endopeptidase Inhibition and Adrenomedullin in Sheep With Experimental Heart Failure


Abstract—Adrenomedullin and the natriuretic peptides exert vasodilator, natriuretic, and aldosterone-inhibitory actions, making augmentation of both systems potential therapeutic strategies in heart failure. Adrenomedullin and an endopeptidase inhibitor (SCH32615) were administered separately and in combination in 8 sheep with heart failure. Compared with the control condition, SCH32615 (5 mg bolus + 1 mg/kg per hour infusion for 3 hours) reduced arterial pressure, left atrial pressure, and peripheral resistance and increased cardiac output, urinary volume, sodium, creatinine, and cAMP excretion. Plasma atrial and brain natriuretic peptide and cGMP concentrations were increased, whereas aldosterone tended to fall. Adrenomedullin (50 ng/kg per minute infusion for 3 hours) induced directionally similar but significantly greater changes in all hemodynamic variables compared with SCH32615. Urinary cAMP, sodium, and creatinine excretion rose, whereas urinary volume was maintained. Circulating adrenomedullin, cAMP, renin, and angiotensin II levels were increased, aldosterone was reduced, and natriuretic peptide levels were unchanged. Co-administration of adrenomedullin and SCH32615 produced hemodynamic effects greater than those achieved during adrenomedullin administration alone. Despite the larger falls in blood pressure, renal function (urinary volume, sodium excretion, and creatinine clearance) was improved to a level similar to that during SCH32615 administration. Elevations in plasma adrenomedullin and cAMP were greater than those during adrenomedullin administration alone, whereas increments in natriuretic peptides were similar to those during SCH32615 alone. Plasma renin and angiotensin II were increased and aldosterone levels were reduced. In conclusion, cotreatment with adrenomedullin and an endopeptidase inhibitor has beneficial hemodynamic and renal effects in heart failure beyond those of either agent separately. (Hypertension. 2002;39:93-98.)

Key Words: adrenomedullin ■ natriuretic peptides ■ enzyme ■ heart failure ■ natriuresis

Adrenomedullin (ADM) and the natriuretic peptides exhibit vasodilator, natriuretic, and aldosterone-inhibitory activities.1–4 These actions are likely to be beneficial in heart failure (HF), making augmentation of both peptide systems a potential therapeutic strategy. ADM and the natriuretic peptides are activated in HF in proportion to the degree of functional and hemodynamic impairment.5,6 Whereas the heart is the predominant site of atrial and brain natriuretic peptide (ANP and BNP, respectively) production,7 vascular tissue (particularly endothelial cells) is likely the major source of circulating ADM in health.8,9 with increased cardiac contribution in HF.10 The modes of action of these peptides differ: cGMP is the intracellular second messenger for ANP and BNP,11 whereas ADM activates receptors coupled to adenylate cyclase with cAMP generation.12 Increasing evidence suggests that there may be interactions between these 2 peptide systems.12,13 In both experimental1 and human HF,13–15 ADM has exhibited beneficial hemodynamic, hormonal, and renal effects. Similarly, augmentation of the natriuretic peptides, via exogenous administration and through prevention of enzymatic degradation by neutral endopeptidase (NEP), has proven to be favorable in the treatment of HF.3,16,17 The present study was designed to test the hypothesis that combined administration of ADM and an NEP inhibitor will produce additional hemodynamic and renal benefits in HF beyond those achieved by either treatment separately.

Methods

Surgical Preparation

Eight Coopworth ewes (38 to 47 kg) were instrumented via a left lateral thoracotomy.18 Under general anesthesia (induced by 17 mg/kg IV thiopentone, maintained with halothane/nitrous oxide), 2 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 cardiac output (CO); a 7F Swan-Ganz...
catheter was inserted in the pulmonary artery for infusions; and a 7F His-bundle electrode was stitched subepicardially to the wall of the left ventricle for left ventricular pacing. A bladder catheter was inserted through the urethra for urine collection. Animals recovered for 14 days before the study protocol was begun. During the experiments, the animals were held in metabolic cages, had free access to water, and ate a diet containing 80 mmol/d sodium and 200 mmol/d potassium.

**Study Protocol**

HF was induced by 7 days of rapid left ventricular pacing (225 bpm) and maintained by continuous pacing for the duration of the study. On 4 separate days (a day apart), the sheep received, in random order, a vehicle control (Haemaccel; Hoechst Marion Roussel, NSW, Australia), human ADM 1-52 alone (50 ng/kg per minute infusion for 3 hours), an NEP inhibitor alone (SCH32615, 5 mg/kg bolus +1 mg/kg per hour infusion for 3 hours), and both agents combined. Infusions were administered in a total volume of 60 mL via the pulmonary artery catheter.

MAP, LAP, CO, and calculated total peripheral resistance (CTPR = MAP/CO) were recorded at 15-minute intervals in the hour before infusion (baseline) and at 15, 30, 45, 60, 90, 120, and 180 minutes during both the 3-hour infusion and postinfusion periods. Hemodynamic measurements were determined by online computer-assisted analysis by use of established methods. Blood samples were drawn from the left atrium at 30 minutes and immediately before infusion (baseline) and at 30, 60, 120, and 180 minutes during the 3-hour infusion and postinfusion periods. Samples were taken into tubes on ice, centrifuged at 4°C, and stored at either −20°C or −80°C before assay for ADM, cAMP, ANP, BNP, cGMP, plasma renin activity, angiotensin II, aldosterone, endothelin-1, catecholamines, and cortisol. Plasma electrolytes and hematocrit were measured in every sample taken. Urinary volumes and samples for the measurement of urinary cAMP, cGMP, sodium, potassium, and creatinine excretion were collected hourly. Creatinine clearance was calculated as urine creatinine/plasma creatinine. Human ADM 1-52 was synthesized as previously described.

The study protocol was approved by the local Animal Ethics Committee.

**Statistical Analysis**

Results 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 (treatment/time interactions quoted in text). Statistical significance was assumed at *P*<0.05.

**Results**

Pacing induced the hemodynamic, hormonal, and sodium-retaining hallmarks of congestive HF, with reduced MAP and CO and increased LAP and hormone levels (see Figures 1 through 5 and Table) compared with normal laboratory measurements (data not shown). There were no significant intergroup differences in pretreatment baseline data.

Compared with control data, all active treatments reduced MAP, LAP, and CTPR and increased CO (all *P*<0.001) (Figure 1). Hemodynamic changes induced by ADM were significantly greater than those induced by SCH32615 (all *P*<0.001). Compared with ADM alone, ADM+SCH32615 further reduced MAP (*P*<0.001), LAP (*P*<0.001), and CTPR (*P*<0.06) and increased CO (*P*<0.05). Hematocrit was increased relative to control by SCH32615 (*P*<0.001) and reduced similarly by both ADM and ADM+SCH32615 (both *P*<0.001) (Table). SCH32615 increased urinary volume (*P*<0.01), sodium excretion (*P*<0.001), potassium excretion (*P*<0.001), creatinine excretion (*P*<0.01), and creatinine clearance (*P*<0.01) compared with the control condition (Figure 2 and Table). ADM increased urinary sodium, potassium, and creatinine excretion and creatinine clearance during and immediately after treatment (all *P*<0.01), whereas urinary volume was unchanged during the infusion and increased significantly afterward (*P*<0.001). ADM+SCH32615 produced significant increases in all renal indexes during as well as after treatment (all *P*<0.001).

Infusion of ADM significantly increased plasma immunoreactive (ir)-ADM (ADM alone and ADM+SCH32615, both *P*<0.001) in association with rises in plasma cAMP (ADM, *P*<0.01; ADM+SCH32615, *P*<0.001) and urinary cAMP (both *P*<0.001) (Figure 3). Despite similar ADM infuse concentrations in both ADM study limbs (ADM alone, 319.0±31.5 nmol/L; ADM+SCH32615, 320.2±23.7 nmol/L), achieved plasma ir-ADM was greater when given in combination with SCH32615 (*P*<0.01). Concordantly, plasma cAMP tended to be greater during combined treatment than during ADM-alone treatment (0.1>P>0.05). SCH32615 alone had no effect on plasma ir-ADM or cAMP. Plasma ANP and BNP and plasma and urinary cGMP levels were increased by SCH32615 (all *P*<0.001). They also rose significantly after cessation of the ADM infusion (all *P*<0.001) (Figure 4). Natriuretic peptide concentrations were matched during ADM and vehicle infusions. Combined ADM
and SCH32615 increased plasma ANP, BNP, cGMP, and urinary cGMP during and after treatment (all $P < 0.001$).

ADM and ADM+SCH32615 increased plasma renin activity (both $P < 0.001$) and angiotensin II ($P < 0.001$ and $P < 0.01$, respectively), with later reductions in plasma aldosterone ($P < 0.05$ and $P < 0.001$, respectively) (Figure 5). SCH32615 tended to reduce plasma aldosterone levels ($P = NS$) but did not alter plasma renin activity or angiotensin II. Plasma potassium was reduced by all active treatments (all $P < 0.05$), whereas plasma creatinine fell with ADM and ADM+SCH32615 (both $P < 0.05$) (data not shown).

No treatment significantly altered plasma endothelin-1, cortisol, or catecholamine (Table) or sodium (data not shown) concentrations.

**Discussion**

This is the first report of the hemodynamic, hormonal, and renal effects of combined administration of ADM and an NEP inhibitor in experimental HF. ADM had much more impact on hemodynamic status than did SCH32615, and cotreatment induced still greater reductions in cardiac preload and afterload and increase in CO. Despite the greater reduction in blood pressure (and hence, renal perfusion pressure), combined treatment improved renal function relative to control to a degree similar to that observed with SCH32615 alone.

**Hemodynamics**

Both ADM and NEP inhibition reduced MAP and LAP and increased CO. The magnitude of change in each case was significantly greater during ADM, although the duration of arterial and atrial pressure effects were more prolonged after NEP inhibition, consistent with the more sustained elevations in plasma CGMP levels compared with cAMP. The natriuretic peptides (during NEP inhibition) lower blood pressure through both vasodilation (as assessed by the fall in CTPR) and a decrease in circulating volume (as judged by the rise in hematocrit). A reduction in venous return may also have contributed to the observed fall in LAP. The hypotensive effect of ADM appears to be primarily due to a direct affect on arterial tone,

Figure 2. Mean±SEM renal responses to 3-hour infusions of vehicle (open bars), ADM (50 ng/kg per minute infusion) (hatched bars), SCH32615 (5 mg/kg bolus +1 mg/kg per hour infusion) (crosshatched bars), and ADM+SCH32615 (solid bars) in 8 sheep with HF. Significant differences between vehicle and the active treatments (calculated by Fisher protected least significant difference tests) are as follows: *$P<0.05$, **$P<0.01$, and †$P<0.001$.

Figure 3. Mean±SEM plasma ir-ADM, plasma cAMP, and urinary cAMP responses to 3-hour infusions of vehicle (○), ADM (50 ng/kg per minute infusion) (●), SCH32615 (5 mg/kg bolus +1 mg/kg per hour infusion) (▲), and the combination of ADM+SCH32615 (■) in 8 sheep with HF.
These results demonstrate the beneficial hemodynamic effects of combined augmentation of ADM and the cardiac natriuretic peptides in HF beyond the effects found with either agent alone.

Renal Function
NEP inhibition increased urinary cGMP in association with a significant natriuresis and diuresis, effects that have been well documented previously. In agreement with earlier studies, ADM increased urinary cAMP and sodium excretion and more than maintained urinary output in the face of larger blood pressure reductions. In addition to any effects of NEP inhibition and ADM administration on tubular reabsorption and renal vascular resistance and increases in renal blood flow, increased glomerular filtration (as assessed by increased creatinine clearance) appears to have contributed to the natriuretic effects observed with both treatments in the present study. The rises in urinary volume and sodium and creatinine excretion that were similar to those observed during NEP inhibition alone (despite significantly greater reductions in MAP) as well as striking postinfusion natriuresis and diuresis values that were greater than those observed after ADM alone. It is remarkable that such an impressive natriuresis occurred during combined treatment in the face of major drops in blood pressure, because the natriuretic effects of ANP and BNP are very sensitive to changes in renal perfusion pressure. It has been suggested by Lisy et al that NEP inhibition potentiates the natriuretic and diuretic responses to intrarenal ADM. These effects were reportedly secondary to a decrease in tubular sodium reabsorption in association with renal vasodilation. Whatever the mechanisms, the ability of combined ADM and inhibition of NEP to increase glomerular filtration, urinary volume, and sodium excretion in the face of such low levels of renal perfusion pressure is remarkable and of potential therapeutic importance.

Hormones
Despite similar ADM infusate concentrations in both ADM study limbs, achieved plasma ir-ADM was greater when ADM was given in combination with the NEP inhibitor SCH32615. This occurred in association with further augmentation of plasma cAMP. This might be interpreted as...
demonstrating an inhibitory effect of NEP inhibition on enzymatic degradation of ADM. Indeed, this could be consistent with data in anesthetized dogs by Lisy et al. showing increased plasma ADM concentrations after dual administration of ADM (intrarenal) and the NEP inhibitor Candoxatrilat (systemic; Pfizer). On the other hand, in vitro studies performed by Lewis et al. found no significant effect of the NEP inhibitor phosphoramidon on the degradation of ADM. It is possible that a product of ADM is formed in vivo, which cross-reacts in the ADM radioimmunoassay and is affected by NEP inhibition. Alternatively, other mechanisms, such as an effect of the natriuretic peptides on ADM secretion or clearance, could play a role in vivo. However, Lainechbury et al. found no effect of coinfusion of BNP with ADM on achieved plasma levels of ir-ADM in patients with HF. It remains possible that NEP inhibition affects ADM clearance indirectly through alterations in the clearance and subsequent bioactivity of one or more of the broad array of substrates for this enzyme. Clearly, further investigations are required.

In agreement with previous investigations of NEP inhibition in HF, we found that plasma natriuretic peptides remained unaltered during ADM infusion, despite the significant fall in LAP (indicating reduced stimulus for secretion and release of these peptides), and were then increased after infusion. These data contrast sharply with those of other studies demonstrating a close parallelism between falls in atrial pressure and plasma natriuretic peptides with the administration of vasodilator agents in HF. Although our findings tend to suggest that ADM directly stimulates natriuretic peptide secretion, an in vitro study has reported suppression of ANP mRNA expression by ADM in neonatal rat cardiocytes. It is possible that ADM modifies some other regulatory mechanism of natriuretic peptide production, such as angiotensin II, which is elevated significantly by ADM and is reported to directly stimulate natriuretic peptide secretion from the heart. Clearly, the interactions between ADM and the natriuretic peptides require further study. Combined treatment increased plasma natriuretic peptides to a similar extent as NEP inhibition alone, despite larger reductions in LAP.

ADM given alone and in combination with SCH32615 stimulated plasma renin activity and angiotensin II, an ex-
pected result given the substantial falls in blood pressure (and hence, renal perfusion pressure) and previous reports of direct stimulation of renin release by ADM.29 Despite the marked rise in plasma angiotensin II, plasma aldosterone levels were reduced by ADM, a finding consistent with these peptide aldosterone-inhibitory actions.2 On the other hand, NEP inhibition was associated with stable plasma renin and angiotensin II levels (in the face of blood pressure reductions) and a tendency for aldosterone levels to fall, observations consistent with the known renin and aldosterone inhibitory actions of the natriuretic peptides.3,4 The inhibition of aldosterone by both the natriuretic peptides and ADM may have contributed to their natriuretic/ diuretic actions.

In conclusion, the present study demonstrates for the first time that combined administration of ADM and an NEP inhibitor has additive acute hemodynamic and renal benefits in experimental HF. Specifically, cotreatment produced significantly greater falls in cardiac preload and afterload and a greater increase in CO than did either treatment separately. Furthermore, combined treatment improved renal function to a level similar to that of NEP inhibition alone, notwithstanding the greater fall in arterial pressure. These data indicate that augmentation of these 2 peptide systems has potential therapeutic importance in clinical intervention for cardiac failure.

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