Bioactivity and Interactions of Adrenomedullin and Brain Natriuretic Peptide in Patients With Heart Failure

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Abstract—Plasma concentrations of the recently discovered hormones adrenomedullin (ADM), from vascular tissue, and brain natriuretic peptide (BNP), secreted by myocardium, are elevated in patients with heart failure. We tested the hypotheses that short-term increments in circulating levels of these hormones, within the pathophysiological range, would have biological effects and that the 2 hormone systems interact. Eight patients with heart failure (left ventricular ejection fractions <35%) received 4-hour infusions of BNP (3.0 pmol ⋅ kg⁻¹ ⋅ min⁻¹) alone, ADM (2.7 pmol ⋅ kg⁻¹ ⋅ min⁻¹ and 5.4 pmol ⋅ kg⁻¹ ⋅ min⁻¹ for 2 hours each) alone, ADM and BNP combined, and placebo. BNP and ADM infusions raised plasma levels of the respective peptide within the pathophysiological range. Arterial blood pressure fell (P<0.05) with all peptide infusions, but cardiac output was unchanged. Heart rate increased with ADM and combined infusions (P<0.01). Sodium excretion rose (P<0.05), and creatinine clearance was sustained during both BNP and combined infusions. Urine volume increased in response to BNP alone (P=0.02). Despite a >2-fold increase in plasma renin with both ADM and combined infusions (P<0.05), plasma aldosterone remained lower than time-matched placebo levels. Plasma noradrenaline was increased by combined, BNP, and higher dose ADM infusions (P<0.05). ADM suppressed plasma cGMP (P<0.05) and inhibited the plasma cGMP response to BNP (P<0.05). The vascular hormones ADM and BNP, produced by myocardium, at plasma concentrations within the pathophysiological range have hemodynamic, renal, and hormonal effects and measurable interactions in patients with heart failure. (Hypertension. 1999;34:70-75.)

Key Words: heart failure ■ adrenomedullin ■ natriuretic peptides ■ arterial pressure

N eurohormonal activation plays a crucial role in the pathophysiology of heart failure. Adrenomedullin (ADM), discovered in 1993, and brain natriuretic peptide (BNP) are peptides with vasodilator, natriuretic, and aldosterone-inhibitory actions. Plasma levels of both peptides are increased in proportion to the degree of hemodynamic compromise. BNP is secreted predominantly from the left ventricle in response to increases in wall stress, whereas production of ADM from cultured endothelial and vascular smooth muscle cells suggests that blood vessels may be a major source of circulating ADM. It is likely that ADM functions in an autocrine or paracrine fashion in addition to any role it may have as a circulating hormone because tissues with ADM receptors are also those with ADM synthetic capability.

Preliminary data suggest that there may be interactions between ADM, produced primarily in the peripheral circulation, and the cardiac natriuretic peptides produced in the heart. Currently, however, there is no information regarding the effects of infused ADM in patients with heart failure and no data directly comparing the biological actions of ADM and BNP. Furthermore, the effects of coadministration of ADM and BNP in humans are unknown. We hypothesized that these peptides alone and in combination would have biological effects and may exhibit interactions in patients with heart failure.

Methods

The experimental protocol was approved by the Southern Regional Health Authority Ethics Committee (Canterbury), and patients gave written informed consent. We studied 8 men aged 53 to 75 years with stable, treated heart failure (ischemic n=5, idiopathic dilated cardiomyopathy n=3). Left ventricular ejection fractions were ≥35% (range 18% to 35%) on 2D echocardiography. All subjects were in NYHA functional class II or III and had no other major disorders or renal impairment. All were clinically stable and on long-term treatment with an angiotensin-converting enzyme inhibitor and furosemide. Five patients were also receiving aspirin and 1 patient was receiving digoxin. None was receiving β-blocker therapy.

Subjects were studied on 4 occasions; on the third and fifth days of 2 periods of constant dietary sodium (100 mmol/d) and potassium (60 mmol/d) intake. The two 5-day diet periods were separated by at least 1 week. Patients were studied in a placebo-controlled, single-blind, crossover fashion receiving in random order a 4-hour intravenous infusion of synthetic human BNP (Bachem, 3 pmol ⋅ kg⁻¹ ⋅ min⁻¹) in vehicle (Hemaccel) (Behring, 10 mL/h); human ADM (Cilnalfa AG, 2.7 pmol ⋅ kg⁻¹ ⋅ min⁻¹ for 120 minutes, and 5.4 pmol ⋅ kg⁻¹ ⋅ min⁻¹ for 120 minutes) in Hemaccel (9 and 18 mL/h); a combination of BNP and ADM in the above doses; or Hemaccel.
On each of the 4 study days, patients ate breakfast, reported to the study room, and completed a 24-hour urine collection at 8:00 AM. All medications were withheld during the previous 24 hours. Patients drank 150 mL of water every 2 hours between 8:00 AM and 3:00 PM and remained seated throughout the study day except for standing to pass urine. Venous cannulas were placed (1 in each forearm) for separate infusion of BNP, ADM, and placebo and for venous sampling. A standard blood pressure cuff was attached to the right arm, and arterial pressure and heart rate were monitored by a noninvasive, fully automated oscillographic sphygmanometer (PP203 MIL, Nippon Co). The mean of 2 consecutive recordings was used in analysis. Cardiac output was recorded with the thoracic impedance method (Minnesota impedance cardiograph model 304B, Instrumentation for Medicine Inc).

Echocardiographic examinations were performed at the end of each infusion phase by an individual technician blinded to the study phase. Measurements included left ventricular volumes and ejection fraction by acoustic quantification and cardiac output by use of the velocity time integrated aortic flow, aortic annulus area, and heart rate. After a 60-minute baseline observation period, infusions commenced. Hemodynamic monitoring and collection of plasma and urine samples were performed as shown in Figure 1.

Venous samples were taken for measurements of ADM, ANP, cGMP, cAMP, aldosterone, plasma renin activity, cortisol, and catecholamines, biochemistry (sodium, potassium, glucose, creatinine, and microhematocrit), hemodynamics (arterial pressure, heart rate, and cardiac output), and urine (sodium, potassium, cGMP, cAMP, and volume).

Results

The patients completed all phases of the protocol without adverse effects, and collection of results was complete. Twenty-four hour urinary sodium, potassium, and creatinine excretion, collected before each of the 4 study days, was not significantly different between study days (data not shown). The baseline plasma BNP level of 25±2.6 pmol/L (Figure 2), was 5-fold the mean for healthy volunteers (4.8±0.2 pmol/L; n=168). A 3-fold increase in plasma BNP was seen with BNP infusion alone and when combined with ADM, (P<0.0001 versus placebo). However, time matched plasma BNP was on average 11.3 pmol/L lower on the combined infusion day during the higher dose of ADM than with BNP alone (P=0.06). Peak plasma ADM levels were similar to those previously observed in our laboratory and by others in patients with decompensated heart failure (87±11 pmol/L, n=35).5,6

The baseline plasma ADM level of 11±1.4 pmol/L (Figure 2) was higher than that of 44 healthy volunteers (6.1±0.3 pmol/L).14 Plasma ADM levels increased similarly and in a dose-dependent fashion during infusion of ADM alone and in combined infusions (P<0.001, Figure 2). The maximum achieved levels of plasma ADM (=30 and ~60 pmol/L for low and high infusion rates, respectively) were in the range we have observed in patients with reduced left ventricular ejection fractions (LVEF ≤30%) subsequent to myocardial infarction (23±2.7 pmol/L, range 8 to 60 pmol/L, n=23), (A.M. Richards, unpublished observations, 1998).

Plasma cGMP was increased by BNP and combined infusions (P<0.005 for both) but was significantly lower when the 2 hormones were combined than with BNP alone (P<0.05, Figure 2). ADM alone suppressed plasma cGMP below time-matched placebo levels (P<0.05 for both doses). Plasma cAMP was significantly increased by the higher infusion rate of ADM versus placebo, whether the peptide was infused alone or in combination with BNP, but was unaffected by BNP alone (Figure 2).

Systolic and diastolic arterial pressures fell with all active treatments (P<0.05 versus placebo) (Figure 3). Greater falls in systolic and diastolic arterial pressure (mean reductions of 14 and 10 mm Hg, respectively) were seen with ADM alone and combined ADM and BNP infusion (16 and 10 mm Hg) versus BNP infusion alone (5 and 5 mm Hg) (P<0.05 for ADM and combined ADM and BNP infusions versus BNP). The fall in arterial pressure with the lower dose of ADM given alone did not reach statistical significance but did so in combination with BNP (mean fall versus placebo of 8 and 4 mm Hg for systolic and diastolic pressures, respectively, P<0.05 for both). For BNP and combined infusions, systolic and diastolic arterial pressure remained significantly lower than time-matched placebo values after infusions were completed (P<0.05). In contrast, arterial pressure returned promptly to time-matched placebo levels after cessation of infusion of ADM alone (Figure 3).
Heart rate was unchanged by BNP infusion but increased with combined and higher rate ADM infusions \((P<0.01)\) for both. Cardiac output was not altered by any active treatment (Figure 3). Echocardiographic measurements of left ventricular volume and ejection fraction are given in the Table. End diastolic volume tended to be lower at the end of each hormone infusion versus placebo \((NS)\) as did end systolic volume \((significant only with infusion of ADM alone, \(P<0.02)\). Ejection fraction was higher with active treatments versus placebo, reaching statistical significance for the BNP infusion \((P<0.01)\). A single echocardiography estimate of cardiac output on each study day revealed no clear difference with hormone infusions versus placebo (Table).

PRA was unaffected by BNP but increased more than 2-fold with the higher dose of ADM and combined infusions \((P<0.05)\). Despite stimulation of PRA, plasma aldosterone was suppressed below time-matched placebo levels during ADM infusion \((P=0.02)\) with the higher dose. Plasma cAMP was unchanged by the lower dose of ADM infusion during ADM and combined infusions but increased by the end of the higher dose \((P=0.05)\).

Heart rate was unchanged by BNP infusion but increased with combined and higher rate ADM infusions \((P<0.01)\) for both. Cardiac output was not altered by any active treatment (Figure 3). Echocardiographic measurements of left ventricular volume and ejection fraction are given in the Table. End diastolic volume tended to be lower at the end of each hormone infusion versus placebo.

**Figure 2.** Plasma BNP, ADM, cGMP, and cAMP responses to infusions of vehicle (placebo □), combined ADM and BNP ●, BNP alone ■, and ADM alone ○. Plasma BNP was significantly increased by infusions of BNP alone and when combined with ADM \((P<0.0001)\). Plasma ADM was significantly increased during both low-dose and higher-dose ADM infusion given alone and when combined with BNP \((P<0.001)\). cGMP was significantly increased by BNP and combined infusions \((P<0.005)\). Plasma cGMP levels were significantly lower with combined ADM and BNP than with infusion of BNP alone \((P<0.001)\). With ADM alone, cGMP was reduced vs placebo \((P=0.004)\). Plasma cAMP was unchanged by the lower dose of ADM infusion during ADM and combined infusions but increased by the end of the higher dose \((P=0.05)\).

**Left Ventricular Volumes and Ejection Fraction by Acoustic Quantification and Cardiac Output at End of Infusion Phase**

<table>
<thead>
<tr>
<th></th>
<th>End Diastolic Volume, mL</th>
<th>End Systolic Volume, mL</th>
<th>Left Ventricular Ejection Fraction, %</th>
<th>Cardiac Output, L/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>255±27</td>
<td>207±25</td>
<td>21±2</td>
<td>5.7±0.4</td>
</tr>
<tr>
<td>Combined</td>
<td>222±31</td>
<td>165±28</td>
<td>27±3</td>
<td>6.1±0.2</td>
</tr>
<tr>
<td>BNP</td>
<td>228±20</td>
<td>170±17</td>
<td>26±2*</td>
<td>5.6±0.4</td>
</tr>
<tr>
<td>ADM</td>
<td>215±25</td>
<td>156±24*</td>
<td>29±4</td>
<td>6.5±0.3</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

*\(P<0.05\) vs placebo.

**Figure 3.** Systolic and diastolic arterial pressure, heart rate, and cardiac output in response to vehicle (placebo □), combined ADM and BNP ●, BNP alone ■, and ADM alone ○. Systolic and diastolic blood pressures were reduced by all active interventions \((P<0.05)\). Greater falls were seen with combined and ADM infusions than with BNP \((P<0.05)\). Heart rate was increased by combined and ADM infusions \((P<0.01)\). Cardiac output was unchanged by any intervention.
noradrenaline was stimulated by combined ADM and BNP infusions (P < 0.001 versus placebo), infusion of BNP alone, and the higher dose of ADM (P < 0.03) (Figure 4). Plasma epinephrine, cortisol, sodium, potassium glucose and creatinine, and the hematocrit were not significantly altered by any peptide infusion (data not shown).

Urinary sodium increased during and after infusion of both BNP alone and combined infusions (P < 0.05) but was unchanged by ADM alone (Figure 5). The difference in urinary sodium excretion in response to BNP infusion and combined ADM/BNP infusion was not statistically significant. Urine volume was increased during the BNP infusion phase only (P < 0.02). Creatinine clearance was similar on all days (Figure 5) as were urinary potassium excretion and urinary cAMP (data not shown). Urinary cGMP was higher during infusion of BNP (127 ± 15 nmol/h) and both hormones (107 ± 18 nmol/h) than placebo (54 ± 11 nmol/h, P < 0.05), but not during infusion of ADM alone (56 ± 13 nmol/h).

**Discussion**

Plasma levels of ADM and BNP are raised in heart failure in proportion to the degree of hemodynamic compromise.6,7 Whereas much is known regarding the secretory patterns and biological effects of BNP under certain circumstances,23 data concerning its actions in heart failure remain sparse.24–26 We chose BNP rather than ANP because plasma levels of the former often reach higher values in the later stages of heart failure yet its bioactivity is less well defined. Even less is known about ADM.3 It is thought to be an autocrine or paracrine vascular peptide, but circulating ADM may also have bioactivity. Regulation of its secretion is poorly understood, and its biological effects in heart failure in man are unknown.

In the present placebo-controlled study, we chose doses of ADM and BNP to attain plasma levels within the pathophysiological range as a first step to understanding their biological effects and interactions. Interpretation of the results should take into account the fact that our patients, of necessity, were receiving long-term treatment with furosemide and an angiotensin converting enzyme inhibitor which, although they were withdrawn 24 hours before each experimental infusion, might alter responses to either hormone.

ADM induced dose-dependent falls in arterial pressure; mean arterial pressure declined by 25%. In contrast, ADM at twice the dose given in the current study induced modest falls in diastolic blood pressure in healthy volunteers.27 Whereas this raises the possibility that ADM may have greater hemodynamic effects in heart failure, Nakamura et al28 reported that the vasodilatory effect of ADM infused into the brachial artery was attenuated in patients with heart failure. Of course, the actions of ADM might differ between vascular beds as
well as between healthy subjects and patients with heart failure.

Although the fall in arterial pressure with BNP infusion was less than that with ADM infusion, the duration of effect was more sustained. This prolonged hypotensive action occurred despite the fact that plasma levels of cGMP, the second messenger for BNP, had returned to placebo levels. Clearly, plasma levels of cGMP do not necessarily reflect tissue bioactivity, at least for the hypotensive action of BNP. With the combination of ADM and BNP, the fall in arterial pressure approximated the sum of the changes induced by each peptide alone, and the prolonged duration of effect of BNP was still evident.

The fall in arterial pressure observed with both ADM and BNP is probably primarily caused by vasodilation. A decline in systemic vascular resistance was seen with ADM infusions in sheep with heart failure and with BNP infusions in human heart failure. Furthermore, as noted above, direct intra-arterial infusion of ADM increased forearm blood flow. Cardiac output was maintained in the present study, and it is unlikely that a reduction in plasma volume could have contributed significantly, because urine volume and hematocrit were little altered.

It has been previously suggested that ADM may blunt the baroreflex-mediated increase in heart rate. Although the present data cannot definitively address this issue, the 30-mm Hg fall in systolic blood pressure with ADM infusion was associated with only a 10-bpm increase in heart rate. Additionally, despite the greater falls in blood pressure with ADM infusion, the increase in plasma noradrenaline was similar to that with BNP.

To our surprise, no change in cardiac output was seen with any infusion. It is possible that the thoracic impedance method used was insufficiently sensitive to detect minor but important changes. An alternative explanation might be that, because the patients we studied were relatively well compensated, balanced decrements in preload and afterload had little effect on cardiac output. In a previous study of similar patients, the same dose of BNP induced no change in cardiac output. Although compelling data in the isolated rat heart indicate that ADM has a positive inotropic action, under the present conditions of study we were unable to define any direct inotropic effects of either hormone.

BNP infusion caused a marked increase in plasma cGMP, whereas ADM induced a modest increase in cAMP, 1 of its proposed second messengers. Despite these obvious differences, ADM induced a greater fall in arterial pressure. Plasma levels of these second messengers may not directly reflect intracellular levels, and it is possible that intracellular cAMP generated by ADM does not enter the extracellular space as readily as cGMP. Alternatively, cell signaling mechanisms other than cAMP may contribute to the hemodynamic actions of ADM.

Adrenomedullin given alone and in combination with BNP induced a marked increase in PRA. This may be expected with the vigorous fall in arterial pressure, a presumed increase in sympathetic flow to the juxtaglomerular apparatus, and perhaps a direct stimulatory action of ADM on renin-secreting cells. Despite the marked increase in PRA, plasma aldosterone levels were suppressed. These observations are consistent with earlier reports of an aldosterone-inhibitory effect of ADM. BNP is capable of inhibiting both renin release and aldosterone secretion, and the lack of change in PRA and aldosterone despite a fall in arterial pressure with BNP infusion is consistent with these known actions.

Although our data do not provide definitive evidence of specific, direct interactions between BNP and ADM, there were trends. Furthermore, clear-cut interactions were observed in the responses of second messenger levels in plasma. ADM infusion tended to reduce plasma BNP levels below time-matched placebo values, and the coinfusion of ADM with BNP gave lower BNP levels than with BNP infusion alone. In keeping with this, plasma cGMP was significantly reduced by ADM infusion alone, and the cGMP response to BNP administration was attenuated by ADM. It might be expected that the hemodynamic changes seen with ADM infusion would inhibit natriuretic peptide secretion (secondary to reduced atrial and ventricular distending pressure), but it should be noted that the lower rate of ADM infusion did not alter arterial pressure, heart rate, or achieved plasma BNP significantly, yet it still reduced BNP-generated cGMP. It is possible that interactions between ADM and natriuretic peptides occur at the level of secretion because ADM inhibits ANP gene expression and peptide secretion in cultured cardiomyocytes. In addition, activation of adenylate cyclase in tissue culture has been shown to increase neutral endopeptidase activity, which would be expected to enhance metabolism of BNP. However, ADM-induced reductions in natriuretic peptide levels were small and nonsignificant, whereas the reductions in plasma cGMP were more substantial, which suggests that ADM in some way impaired the ability of the natriuretic peptides to generate second messenger. A final possibility is that interactions between second messengers at the level of cyclic nucleotide phosphodiesterases accounted for the attenuated cGMP response to BNP when coinfused with ADM. Such interactions require further study, especially because other workers, using bovine aortic endothelial cells, reported ADM stimulated (rather than suppressed) cGMP through activation of nitric oxide synthase.

BNP induced a natriuresis and diuresis despite a minor fall in renal perfusion pressure, as has been noted previously. Although ADM did not induce natriuresis or diuresis, urine volume, sodium excretion, and creatinine clearance were maintained despite marked falls in renal perfusion pressure. The natriuresis observed with combined ADM and BNP infusion in response to low arterial pressure is of interest because the natriuretic effects of BNP and ANP are very sensitive to changes in renal perfusion pressure. It is possible that ADM augmented the BNP-induced natriuresis, but experiments using alternative hypotensive agents together with BNP are needed to clarify this issue.

Results from this study, performed under rigorous experimental conditions, indicate that BNP and ADM at levels within the pathophysiological range have hemodynamic, hormonal, and renal actions in patients with heart failure. Most of these effects are likely to be beneficial, although longer-term studies with larger numbers of patients are now required. Interactions between the 2 hormones, 1 generated by the heart, the other mainly within vasculature, are evident.
Acknowledgments

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References


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