Hypotensive and Natriuretic Actions of Adrenomedullin in Subjects With Chronic Renal Impairment

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Abstract—Plasma levels of adrenomedullin are increased in chronic renal failure. The significance of this finding is uncertain, because the biological effects of adrenomedullin in renal impairment are unknown. Therefore, we studied the effects of adrenomedullin infusion in subjects with chronic renal impairment. Eight males with IgA nephropathy and plasma creatinine of 0.19±0.03 mmol/L (mean±SEM) were studied in a vehicle-controlled crossover design. Each subject was studied twice; subjects were administered either adrenomedullin at a low dose and then a high dose (2.9 and 5.8 pmol/kg per minute, respectively, for 2 hours each) or a 4-hour vehicle control (Hemaccel), in random order, on day 4 of controlled metabolic diets. Adrenomedullin infusion achieved plasma adrenomedullin concentrations in the pathophysiological range after the low (31.2±5.1 pmol/L) and high (47.4±4.3 pmol/L) dose, and plasma cAMP was increased. Compared with vehicle control, high-dose adrenomedullin increased peak heart rate (121.7±3.3 bpm, \( P<0.01 \)) and cardiac output (47.4±4.3 pmol/L) dose, and plasma cAMP was increased. Compared with vehicle control, high-dose adrenomedullin increased peak heart rate (+21.7±3.3 bpm, \( P<0.01 \)) and lowered both systolic and diastolic blood pressures by >10 mm Hg (\( P<0.05 \)). Plasma renin activity, angiotensin II, and norepinephrine increased by up to 50% above baseline levels (\( P<0.05 \) for all), whereas aldosterone and epinephrine were unchanged. Urinary volume and sodium excretion increased significantly (\( P<0.05 \)) with low-dose adrenomedullin, whereas creatinine clearance was stable, and proteinuria tended to decrease. In subjects with chronic renal impairment due to IgA nephropathy, adrenomedullin infusion lowered blood pressure, stimulated sympathetic activity and renin release, and caused diuresis and natriuresis. Adrenomedullin may have a role in modulating blood pressure and kidney function in renal disease. (Hypertension. 2001;37:1279-1284.)

Key Words: adrenomedullin ■ renal disease ■ immunoglobulins ■ proteinuria ■ renin ■ angiotensin II ■ sympathetic nervous system

Adrenomedullin (AM), a potent vasodilator peptide discovered in pheochromocytoma tissue in 1993, is synthesized and secreted mainly by endothelial cells and vascular smooth muscle cells and has been isolated from a variety of tissues. In health, AM circulates in low picomolar concentrations in plasma, but the levels are elevated 2- to 3-fold in chronic renal failure (CRF). It is unclear whether this reflects increased secretion (renal or extrarenal) or reduced clearance of AM from the circulation. In animal studies, AM has important effects on renal function, including increasing renal blood flow (RBF), urinary output, and sodium excretion and inhibiting glomerular mesangial cell (MC) proliferation in vivo.

The few studies that have been performed in humans demonstrate that AM has powerful vasodilator actions and interacts with other neurohormonal systems. Notably, its actions differ between pathophysiological states. With normal subjects, whereas in a recent study, AM produced greater vasodilation in subjects with hypertension than in normal individuals. To date, no studies have examined the actions of AM infusion in human subjects with renal impairment. This is pertinent, given the possibility that the biological effects of AM may be altered in the setting of renal disease. For example, endogenous inhibitors of NO synthase accumulate in CRF, and inhibition of NO synthesis has been shown to attenuate renal responses to AM administration in dogs. Furthermore, the renal expression of AM receptor activity-modifying proteins 1 and 2 is upregulated in rats with CRF because of obstructive nephropathy, which might allow enhanced renal responses.

In the present study, we examined the effects of AM infusion in men with mild chronic renal impairment (CRI) due to IgA nephropathy. We hypothesized (1) that AM would have significant effects on hemodynamic, hormonal, and renal parameters with normal subjects, whereas in a recent study, AM produced greater vasodilation in subjects with hypertension than in normal individuals. To date, no studies have examined the actions of AM infusion in human subjects with renal impairment. This is pertinent, given the possibility that the biological effects of AM may be altered in the setting of renal disease. For example, endogenous inhibitors of NO synthase accumulate in CRF, and inhibition of NO synthesis has been shown to attenuate renal responses to AM administration in dogs. Furthermore, the renal expression of AM receptor activity-modifying proteins 1 and 2 is upregulated in rats with CRF because of obstructive nephropathy, which might allow enhanced renal responses.

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in human subjects with CRI, and (2) that the actions of AM in CRI would differ from those seen in the healthy volunteers and hypertensive subjects studied previously.

Methods
This single-blind, vehicle-controlled, crossover trial was approved by the Canterbury Ethics Committee, and all participants gave written informed consent. Eight white male volunteers with renal biopsy–proven IgA nephropathy were studied. They were aged 42.4±3.4 (mean±SEM) years (range 31 to 57 years). Inclusion criteria were systolic blood pressure (BP) <180 mm Hg or diastolic BP <110 mm Hg after withdrawal of antihypertensive drugs for 2 weeks, mild to moderate CRI (plasma creatinine 0.12 to 0.4 mmol/L), moderate albuminuria (0.4 to 3.5 g/d), little or no anemia (hemoglobin >100 g/L on no erythropoietin), and the absence of other significant medical conditions. All patients were taking 1 (n=6) or ≥2 (n=2) antihypertensive drugs, namely, ACE inhibitors (n=6), losartan (n=1), felodipine (n=2), doxazosin (n=2), and furosemide (n=1). All drugs were withdrawn 2 weeks before starting the study, and the patient’s BP was observed to ensure that it remained within acceptable limits.

Subjects were studied on 2 days separated by a 2-week interval. On the study days, subjects received either a 4-hour infusion of human AM (Clinalfa AG) in 50 mL Hemaccel (Hoescht Marion Roussel, Australia) at a low (2.9 pmol/kg per minute) and then a high (5.8 pmol/kg per minute) dose for 2 hours each or a 4-hour infusion of vehicle control (50 mL hemaccel alone). The sequence of infusions (AM or vehicle) was randomized, and both were administered on day 4 of a controlled diet containing 80 mmol sodium and 100 mmol potassium daily. On both experimental days, the patients ate a caffeine-free breakfast at 7:45 AM and completed a 24-hour urine collection at 8:00 AM for measurements of urinary volume, of sodium, potassium and protein excretion, and of endogenous creatinine concentration. Venous cannulas were placed in the dominant forearm for blood sampling and in the nondominant hand for infusion of AM or vehicle.

Infusions ran continuously from 10:00 AM to 2:00 PM. The patients remained seated in an easy chair until 3:00 PM but stood briefly on 4 occasions (before infusion, at the end of the low- and high-dose phases, and at the completion of the study) to pass urine. Arterial pressure and heart rate were recorded in duplicate every 30 minutes by using an automated oscillometric sphygmomanometer (PP203 MII, Nippon Column Co), and cardiac output was measured by the thoracic impedance method (Minnesota Impedance Cardiograph 304B, Instrumentation for Medicine Inc).

Neurohormones
Venous samples were drawn before (2 times) and every 30 minutes during and after (3 times) each infusion for plasma creatinine, sodium, and potassium levels (Aeroset, Abbott Diagnostics) and neurohormonal measurements. The neurohormonal samples were centrifuged immediately, and the separated plasma was stored at −80°C until analysis. The hematocrit was measured in duplicate at the beginning and end of each infusion. Epinephrine and norepinephrine were measured by liquid chromatography with electrochemical detection. As previously described, radioimmunoassay was used for measurements of AM, atrial natriuretic peptide, brain natriuretic peptide, aldosterone, renin activity, and cAMP (commercial kit, Biotrak, Amersham); cortisol was measured by ELISA. Plasma prolactin (Beckman Access analyzer) was measured hourly, and samples for plasma angiotensin II (Ang II) were collected every 2 hours into tubes containing EDTA and Enalkiren (Abbott Laboratories) and analyzed by radioimmunoassay. All plasma samples from an individual were analyzed in a single assay to avoid interassay variability. Intra-assay coefficients of variation fell between 5% and 9%.

Urinary Measurements
Urinary volumes were recorded, and samples were analyzed for sodium and creatinine concentrations (Hitachi autoanalyzer), total protein (dye-binding method), and urinary albumin and IgG (both by an ELISA technique). Retinol binding protein concentration was determined by a double-antibody sandwich ELISA. Urinary protein results were expressed as urinary concentration (g/L), total excretion, and the protein/creatinine concentration ratio over each time interval.

Statistical Analysis
Data were analyzed with SPSS statistical software by 2-way ANOVA, with treatment and time as repeated measures. Where a significant effect was seen in any phase, changes from baseline to a given time point in the AM versus vehicle phases were compared by paired t tests. A value of P<0.05 (2-tailed) was considered to indicate statistical significance. Graphs were drawn with Sigma plot, and results are presented as mean±SEM.

Results
All 8 subjects completed the study, and data collection was complete. AM infusion was well tolerated, with mild facial flushing in 6 subjects and mild headache in 3 subjects. Baseline clinical and urinary factors were closely matched between placebo and AM study days (P=NS for all, Table).

Neurohormonal Effects
Plasma AM concentrations were similar at baseline on both experimental days (8.8±1.2 pmol/L on the AM day and 9.1±1.2 pmol/L on the vehicle day) and remained stable throughout vehicle infusion. There were correlations between baseline AM level and creatinine clearance (r=-0.43, P=0.29) and 24-hour albumin excretion (r=0.57, P=0.14) that did not reach statistical significance. Plasma AM levels increased significantly during low-dose (peak 31.2±5.1 pmol/L, P<0.001) and high-dose (peak 47.4±4.3 pmol/L, P<0.0001) AM infusion and were accompanied by a rise in plasma cAMP (Figure 1). Plasma prolactin levels were higher during high-dose AM infusion than during vehicle infusion, and cortisol levels were increased compared with those during vehicle infusion at the completion of the AM infusion (Figure 1). Baseline atrial natriuretic peptide (16.9±3.2 pmol/L) and brain natriuretic peptide (7.8±2.0 pmol/L) were in the normal range and did not change with AM infusion (data not shown). Compared with time-matched control data, plasma renin activity and Ang II levels increased during high-dose AM infusion, whereas aldosterone was unchanged. Norepinephrine levels increased during high-dose AM by >50% above baseline, but epinephrine levels were unchanged (Figure 2).
Hemodynamic Effects
High-dose AM infusion increased heart rate by up to 21.7 ± 3.3 bpm above the time-matched control ($P < 0.01$). BP was stable during low-dose AM infusion, but systolic ($-11.3 ± 1.8$ mm Hg, $P < 0.05$) and diastolic ($-11.8 ± 2.9$ mm Hg, $P < 0.05$) pressures fell during the higher dose (Figure 3). Cardiac output was stable during vehicle infusion but increased ($+2.9 ± 0.2$ L/min, $P < 0.01$) during high-dose AM administration. Heart rate remained elevated 1 hour after AM infusion ($P < 0.05$), whereas BP and cardiac output returned promptly to time-matched control values.

Renal Effects
Urinary volume increased by $1.1 ± 0.3$ mL/min ($P < 0.05$), and urinary sodium excretion increased by $50 ± 7$ μmol/min ($P < 0.05$) during low-dose AM compared with vehicle control, but both were similar to vehicle during and after high-dose AM (Figure 4). These results remained consistent when corrected for endogenous creatinine clearance. Creatinine clearance and potassium excretion were similar across the 2 experimental days. Urinary total protein, albumin, IgG, and retinol binding protein excretion were lower with AM than with vehicle, but differences were not statistically significant (Figure 4).

Discussion
Several lines of evidence point to a role for AM in the maintenance of renal function. Immunoreactive AM and its mRNA have been found in renal tubular cells, MCs, and vascular smooth muscle cells of experimental animals and in cultured human endothelial cells and MCs. In rat studies, AM receptors and/or mRNA was also present, including in MCs, renal vessels, inner medullary collecting ducts, and distal convoluted tubules, suggesting that AM may have an autocrine or paracrine role in the regulation of renal glomerular and tubular function.

Renal AM levels increase with hypoxia, with arginine vasopressin or tissue necrosis factor administration, and with experimental heart failure, suggesting that AM may contribute to renal responses in a variety of pathophysiological states. Animal studies demonstrate that AM has important renal actions. AM administration increases cAMP in renal tubules and in rat cultured MCs. AM also reduces MC mitogenesis, proliferation, and endothelin production and inhibits Ang II–induced progression of renal fibrosis by reducing fibroblast proliferation and extracellular matrix production. Furthermore, AM stimulates renin secretion by juxtaglomerular cells, an action that may also be mediated via cAMP. In experimental animals, intravenous AM lowered BP, increased heart rate, urinary volume, and sodium excretion, and increased RBF with little or no effect on glomerular filtration rate. Intrarenal administration of...
AM has been shown to produce natriuresis as a result of reduced tubular sodium resorption.\textsuperscript{34–36}

However, there is little information regarding the pathophysiological role of AM in human subjects with decreased renal function. Cross-sectional studies in CRF have found that AM levels are positively correlated with plasma creatinine and proteinuria and, in chronic glomerulonephritis, with biopsy evidence of disease activity.\textsuperscript{37,38} The biological effects of raising plasma AM levels in patients with CRI are unknown.

In the present study, we demonstrate that human exogenous AM\textsubscript{1–52} infusion in men with IgA nephropathy and CRI produces vasodilatation, lowers arterial pressure, increases heart rate and cardiac output, and stimulates the renin-angiotensin system and norepinephrine levels, whereas plasma aldosterone and epinephrine concentrations are unchanged. We have also demonstrated for the first time in human subjects that in this setting, AM has natriuretic and diuretic actions. The BP-lowering effects observed are similar to those seen in earlier animal and human studies and likely reflect the powerful vasodilator action of AM mediated through NO- and cAMP-dependent pathways.\textsuperscript{2,7} The increase in heart rate and cardiac output may reflect, in part, compensatory baroreceptor-mediated sympathetic activation.\textsuperscript{2} In addition, AM has been shown to have direct positive inotropic actions in an isolated heart model,\textsuperscript{39} ie, actions that directly stimulate heart rate and cardiac output in sheep\textsuperscript{40} and increase sympathetic activity in rabbits by central mechanisms.\textsuperscript{41}

Activation of the renin-angiotensin system has been demonstrated in earlier studies\textsuperscript{7} and likely occurs via several mechanisms, including reduced renal perfusion pressure, sympathetic activation, and direct stimulation of renin secretion.\textsuperscript{2,31}

The dose of AM used in the present study was chosen to achieve plasma levels seen under pathophysiological conditions, particularly severe CRF,\textsuperscript{3} but also after myocardial infarction,\textsuperscript{42} congestive heart failure,\textsuperscript{43} and sepsis.\textsuperscript{2} During low-dose AM, BP was unchanged from that during placebo (Figure 3), despite the fact that plasma AM levels were well into the pathophysiological range (Figure 1). Notably, during low-dose AM, urinary output and sodium excretion increased (Figure 4). The natriuretic or diuretic effect of AM was lost during high-dose infusion, when systolic and diastolic BP fell by >10 mm Hg. These results suggest that any natriuretic action of AM may be sensitive to perfusion pressure, as is the case with other hormonal systems, including the natriuretic peptides.\textsuperscript{44} Natriuresis has been demonstrated in animal models with intrarenal administration of AM, when systemic pressure is not altered.\textsuperscript{34}

The fact that urinary output and sodium excretion increased but that endogenous creatinine clearance was unchanged might indicate a direct action of AM on tubular function. However, it is likely that both glomerular function and RBF would have been altered.\textsuperscript{34,36} In this regard, the
present study would have been strengthened by measures of glomerular filtration rate and RBF by using inulin and p-aminohippurate clearance methods. Of note, intrarenal AM administration in animal studies has produced renal arterial vasodilatation and increased RBF associated with small increases in glomerular filtration rate. Although not statistically significant, urinary protein excretion tended to decrease with AM. This may have been due to a reduction in glomerular pressure and is consistent with the concept that AM may modulate the actions of Ang II on the glomerulus. Clearly, further human studies are required to examine the specific effects of AM on renal hemodynamic and tubular function.

Comparison With Earlier Studies
We used an identical design in previous studies of AM infusion in normal volunteers (NV group) and subjects with uncomplicated essential hypertension (HT group). Subjects in the present study were matched with earlier HT subjects in terms of age, body mass index, and baseline BP but were significantly older and had higher baseline BP than subjects in the NV group. Baseline creatinine clearance was significantly reduced in the CRI group compared with both the NV (162±30 mL/min, P<0.05) and HT (132±16 mL/min, P<0.05) groups. Notably, baseline AM levels were significantly higher in the CRI group than in the NV or HT group (8.9±1.1 versus 6.3±0.6 and 5.4±0.9 pmol/L, respectively; P<0.05 for CRI group versus NV and HT groups). Despite achieving similar peak plasma AM levels in all 3 groups, we observed different hemodynamic and urinary effects in the present study, likely reflecting the unique pathophysiology of CRI. The peak fall in systolic BP (~11±4 mm Hg) with AM infusion in the present study was attenuated compared with that in the HT group (~24±2 mm Hg, P<0.05), although it was similar to that seen in NV group (~6.6±5.1 mm Hg, P=0.3). A similar pattern was evident for diastolic BP. The BP-lowering effect of AM may be attenuated in CRI, possibly as a result of the inhibitory effects on NO synthase in CRI described above. Whereas urinary output and sodium excretion were higher during low-dose AM in the CRI group (Figure 4), these parameters were identical during AM and placebo infusions in the NV and HT groups.

The natriuretic action of AM may reflect changes in hemodynamic and renal responses or altered thresholds for the hemodynamic and renal actions of AM in this setting. Attenuation of NO-mediated vasodilator actions of AM in these subjects may have maintained renal perfusion pressure at a time that intrarenal AM levels crossed the threshold for its glomerular or tubular effects, thereby facilitating natriuresis. In addition, there may be upregulation of receptor activity–modifying proteins within the kidney in CRI, an effect seen in an animal model of obstructive uropathy, which may possibly enhance renal responsiveness to AM, resulting in natriuresis.

The above comparisons of data from AM infusions in NV, HT, and CRI subjects should be viewed as preliminary, because although the study protocols were identical, they were performed in series (NV first, HT second, and then CRI) rather than in parallel; hence, we cannot exclude the effects of season or time of year, for example.

We studied only men with IgA nephropathy. Whereas this gave us a uniform group of subjects, it is unclear whether the results can be extrapolated to patients with other kidney disorders. Further studies are required to expand on our preliminary findings and to elucidate more fully the effects of AM on renal function in humans.

In summary, short-term AM infusion reaching pathophysiological plasma AM levels had significant effects in men with IgA nephropathy and CRI. We confirm that AM lowers arterial pressure in humans and that it has important interactions with the sympathetic and renin-angiotensin systems and aldosterone. We have demonstrated for the first time that AM has diuretic and natriuretic properties in CRI. These findings suggest that AM may contribute to hemodynamic, neurohumoral, and renal responses in CRF. Some actions of AM demonstrated in the present study may be of therapeutic value in hypertension and renal disease, including vasodilator/hypotensive effects, inhibition of the aldosterone response to endogenous angiotensin II, and diuresis/natriuresis. Further studies using long-term infusions, selective blockade, or augmentation of AM are needed to further clarify the role of AM in humans and to evaluate therapeutic applications.

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