Hemodynamic, Hormone, and Urinary Effects of Adrenomedullin Infusion in Essential Hypertension

Richard W. Troughton, Lynley K. Lewis, Timothy G. Yandle, A. Mark Richards, M. Gary Nicholls

Abstract—We examined the effects of the vasodilator peptide adrenomedullin (AM) infused intravenously into subjects with essential hypertension. Eight men 39 to 58 years old with uncomplicated hypertension (147±9/6±5/3 mm Hg at baseline) were studied in a placebo-controlled, crossover design. Each subject received intravenous AM in a low and a high dose (2.9 and 5.8 pmol·kg⁻¹·min⁻¹ for 2 hours each) or vehicle-control (Hemaccel) infusion in a random order on day 4 of a controlled metabolic diet (80 mmol/d Na⁺, 100 mmol/d K⁺). Plasma AM reached pathophysiological levels during infusion (18±4 pmol/L in low dose, 34±9 pmol/L in high dose) with a concurrent rise in plasma cAMP (+8.4±1.2 pmol/L, P<0.05 compared with control). Compared with control, high-dose AM increased peak heart rate (+17.8±2.3 bpm, P<0.01), lowered systolic (−24.6±0.9 mm Hg; P<0.01) and diastolic (−21.9±1.4 mm Hg; P<0.01) blood pressure, and increased cardiac output (+1.0±0.1 L/min in low dose, +2.9±0.2 L/min in high dose; P<0.01 for both). Despite a rise in plasma renin activity during high dose (P<0.05), aldosterone levels did not alter. Plasma norepinephrine levels increased 1295±68 pmol/L (P<0.001) and epinephrine increased 74±15 pmol/L (P<0.05) with high-dose AM compared with control. AM had no significant effect on urine volume and sodium excretion. In subjects with essential hypertension, the intravenous infusion of AM to achieve pathophysiological levels produced significant falls in arterial pressure, increased heart rate and cardiac output, and stimulated the sympathetic system and renin release without concurrent increase in aldosterone. Urinary parameters were unaltered. Although AM has potent hemodynamic and neurohumoral effects in subjects with essential hypertension, the threshold for urinary actions is set higher. (Hypertension. 2000;36:588-593.)

Key Words: hypertension, essential ▪ adrenomedullin ▪ renin ▪ nervous system, sympathetic ▪ blood pressure ▪ cardiac output

Adrenomedullin (AM) is a 52-amino-acid vasodilator peptide isolated in 1993 from human pheochromocytoma. The AM gene, located on chromosome 11 in humans, is expressed in numerous tissues, and the peptide itself appears ubiquitous presumably because high rates of gene transcription and synthesis occur in vascular smooth muscle cells (VSMCs) and endothelial cells. Available studies, although far from being definitive, suggest that AM may contribute to homeostatic responses, at least in sepsis and some cardiovascular disorders. Its potent vasodilator action is well documented and is likely mediated through NO- and cAMP-dependent mechanisms. AM is present in the central nervous system and may have a role in the modulation of salt appetite, thirst, and sympathetic activity. In some models, AM increases glomerular filtration rate and has a natriuretic action. AM also interacts with other hormones; for example, inhibiting the secretion of aldosterone in the adrenal zona glomerulosa and endothelin-1 in VSMCs. It is possible, but unproved, that AM plays a role in the pathophysiology of hypertension (HT). Indeed, plasma levels of AM are elevated in HT in proportion to the severity of blood pressure (BP) elevation and to the degree of renal impairment and correlate with the degree of cardiac and arterial hypertrophy. Studies in animal models of HT have demonstrated that short- and long-term AM infusion lowers arterial BP as does gene delivery of human AM gene in spontaneously hypertensive rats. AM inhibited hypertrophy of cultured cardiomyocytes in 1 study and increased red cell membrane fluidity in another. Because the effects of AM in human hypertensive subjects are unknown, we studied responses to low-dose, short-term AM administration in subjects with essential HT.

Methods

The protocol was approved by the Canterbury Ethics Committee. Eight male volunteers (38 to 58 years) with uncomplicated essential HT, normal renal function, and no other medical problems were enrolled in this placebo-controlled, crossover design study. Subjects were all taking 2 antihypertensive medications. Seven of the 8 were receiving an ACE inhibitor. All medications were stopped 2 weeks before the study and restarted at its completion. Each subject ate a diet with constant sodium (80 mmol/d) and potassium.
(100 mmol/d) content for 4 days before the infusion of AM or vehicle. On both experimental days, the volunteers ate breakfast at 7:45 AM; completed a 24-hour urine collection at 8 AM for sodium, potassium, and creatinine measurement; and remained seated in an easy chair until 3 PM except when standing to urinate (see later). Subjects received 10 mL/kg distilled H2O at 8 AM as a volume load and an additional 200 mL every hour during the study. Venous cannulas were placed in each forearm: 1 for the infusion of AM or vehicle control; and the other for blood sampling. At 9:30 AM, the AM in Hemaccel was infused at 2.9 pmol · kg⁻¹ · min⁻¹ for 120 minutes and then at 5.8 pmol · kg⁻¹ · min⁻¹ for an additional 120 minutes. Alternatively, vehicle alone (50 mL Hemaccel over 240 minutes) was administered. Subjects were blinded as to which infusion was given: 4 received AM first and 4 received the vehicle. Venous samples were drawn before, during, and after each infusion for measurements of AM, plasma renin activity (PRA), and plasma levels of aldosterone, angiotensin II, norepinephrine and epinephrine, cAMP (commercial kit Biotrak; Amersham), brain natriuretic peptide (BNP), atrial natriuretic peptide (ANP), and cortisol.30 Venous blood was also taken on 6 occasions for the measurement of plasma prolactin levels (Access analyzer; Beckman Instruments). All samples from an individual were analyzed in a single assay. Intra-assay coefficients of variation were all <9%. Venous samples were also drawn for measurements of plasma sodium and potassium and for hematocrit determination before and at the completion of low- and high-dose AM and vehicle infusion phases.

On both infusion days, arterial BP and heart rate (HR) were recorded in duplicate at 30-minute intervals with an automatic sphygmomanometer (Electronics Services Limited). On 4 occasions (preinfusion, end of low dose, end of high dose, and completion of study) and after venous sampling, the subjects stood to pass urine for measurements of cAMP, aldosterone, sodium, potassium, and creatinine. Cardiac output was measured with the thoracic impedance method (Minnesota Impedance Cardiograph model 304B; Instrumentation for Medicine Inc).

Human 52-amino-acid AM for infusion was purchased from Clinalfa AG. Results were analyzed with 2-way ANOVA with treatment and time as repeated measures. Where a significant effect was seen in any phase, individual time points were analyzed with an ANOVA method that compared the difference between AM and vehicle control phases in the change from baseline to a given time point. A value of P<0.05 was taken to indicate statistical significance. Results are presented as mean±SEM.

**Results**

Data collection was complete. Six of the 8 subjects described mild headache and exhibited facial and conjunctival injection during high-dose AM infusion (Figure 1). These effects were readily tolerated and resolved rapidly at the end of infusion. Achieved AM infusate concentrations (mean 811±108 pmol/L) were >50% of predicted concentration in 6 of the subjects and ≈25% of predicted concentration in the first 2 subjects.

**Neurohormonal Effects**

Plasma AM levels at baseline were <10 pmol/L on both infusion days. During vehicle infusion, AM levels remained steady, whereas on the day of peptide infusion, levels increased to 18±4 and 34±9 pmol/L at the completion of the 2 infusion rates. AM levels then declined but remained significantly above time-matched vehicle values at 90 minutes after completion of the infusion (Figure 2). Plasma levels of cAMP were unaltered by the lower dose of AM but increased by >5 pmol/L during the higher infusion rate and then fell toward time-matched control values (Figure 2). Plasma BNP levels were similar for the 2 experimental phases. Plasma ANP levels were higher, but not significantly so, during both phases of AM than for the vehicle control (Figure 2). Plasma prolactin levels increased in a dose-dependent fashion with AM infusion (P<0.05; Figure 2).

PRA increased with high-dose AM to 50% greater at peak than the time-matched control levels (P<0.05; Figure 3). There was a nonsignificant rise in plasma angiotensin II levels during high-dose AM. Plasma aldosterone levels tended to be lower with AM than with vehicle infusion (NS). Cortisol levels were matched during both infusion phases and rose (NS) after completion of the AM infusion. Plasma levels of both norepinephrine and epinephrine exhibited a dose-dependent increase with AM (P<0.05 and P<0.001, respectively) and thereafter declined toward time-matched vehicle levels (Figure 3).

**Hemodynamic Effects**

Heart rate increased in a dose-dependent manner with AM administration to 18 bpm higher than vehicle control levels at peak dose (P<0.01), before declining promptly to time-matched vehicle values (Figure 4). Mean systolic BP fell by a maximum of 11 mm Hg with low-dose AM (P<0.05) and by 24.6 mm Hg with high-dose AM (P<0.001). Diastolic BP also exhibited a dose-dependent decline with AM of 9.6 mm Hg during low-dose AM (P<0.05) and 21.9 mm Hg during high-dose AM (P<0.001; Figure 4). Cardiac output showed a dose-dependent increase with AM (P<0.01), especially during the higher dose but also to a significant degree toward the end of the low-dose infusion (Figure 4). The peak
difference between the 2 experimental days was ≈3 L/min. Cessation of AM infusion resulted in a rapid fall in cardiac output (CO) to time-matched control values (Figure 4). Calculated total peripheral resistance (TPR) fell by a peak of 1002 ± 220 dynes · s⁻¹ · cm⁻⁵ (55% of baseline) during high-dose AM (P < 0.001).

Renal Effects
Urine volume, sodium, and potassium excretions were not significantly different between AM and vehicle infusion days (Table) regardless of whether data were corrected for creatinine excretion. Likewise, change and percent change in urine volume, urine sodium, and urine potassium were not significantly different between experimental days. Plasma sodium, potassium, and creatinine and hematocrit were similar on both experimental days and were not altered by AM (data not shown).

Comparison With Normal Volunteers
In a previous study of normotensive volunteers, we used an identical study design and infused AM at the same rates. Although the normal subjects were younger men (24 ± 6 years; P < 0.001), plasma AM levels were similar at baseline (6.3 ± 0.6 versus 5.4 ± 0.9 pmol/L; P = 0.42) and peak (42.4 ± 7.3 versus 33.8 ± 9.0 pmol/L; P = 0.47). Compared with normotensive volunteers, hypertensive subjects experienced similar increases in HR and CO but greater falls in systolic and diastolic BP and TPR. The peak difference in systolic BP between active and vehicle control phases was 18.5 ± 3.0 mm Hg for the hypertensive group compared with 5.1 ± 2.1 mm Hg for the normotensive group (P = 0.003), and the difference for diastolic BP was 19.3 ± 2.4 versus 7.6 ± 1.3 mm Hg (P = 0.002). The percentage falls in systolic BP (13 ± 6% versus 4 ± 6%) and diastolic BP (20 ± 8% versus 11 ± 5%) were greater in the hypertensive than the normotensive subjects (P = 0.009 and P = 0.014, respectively). Calculated TPR was higher at baseline in the HT group (1680 ± 170 versus 980 ± 60 dynes · s⁻¹ · cm⁻⁵; P = 0.02) and fell by a greater amount (1002 ± 220 versus 580 ± 100 dynes · s⁻¹ · cm⁻⁵; P = 0.11). When data from the subjects in each of the 2 studies were combined, there was a close positive relationship between the magnitude of fall in BP with AM infusion and baseline systolic and diastolic BP (r = 0.54, P = 0.034 for systolic; r = -0.64, P = 0.007 for diastolic; Figure 5) and with age (r = -0.68; P = 0.004) but not with achieved AM level (r = -0.151; P = 0.58) or other clinical, biochemical, or hormone variables. With the Oldham transformation applied, the correlation remained significant for diastolic (r = -0.54; P = 0.033) but not systolic (r = -0.40; P = 0.124) BP.
peak fall in TPR was closely related to baseline TPR ($r=0.9, P=0.005$; Figure 5).

**Discussion**

AM is a potent vasodilator peptide.\(^1,15\) Studies in animal models indicate that AM induces a significant fall in BP when administered either by infusion or as a bolus.\(^1,27\) The fall in BP was greater in the spontaneously hypertensive rat than in normotensive animals.\(^26\) Although the exact mechanisms by which AM produces vasodilatation are not defined, there is evidence that it acts indirectly through NO and through cAMP-mediated mechanisms and via the inhibition of angiotensin II and endothelin production.\(^20,32–34\) AM in animals also acts in the kidney, relaxing both efferent and afferent glomerular arterioles, increasing glomerular filtration, and inducing natriuresis.\(^32,33\) In addition, AM may inhibit VSMC and cardiac muscle hypertrophy.\(^28,35\)

To date, no studies have examined the effect of pathophysiological levels of AM in either animal or human models of HT. In the present study, we demonstrate, for the first time, the effects of intravenous AM infusion in human subjects with essential HT. Plasma levels of AM achieved during infusion were within the range seen in acute myocardial infarction,\(^16\) cardiac failure,\(^12\) severe HT, and renal failure.\(^23\) AM administration was accompanied by a significant rise in plasma levels of cAMP during the high dose. Most of our subjects, as with the healthy volunteers in the study of Meeran et al,\(^36\) developed skin flushing during AM infusion, a reflection no doubt of increased skin blood flow.\(^15\) In addition, elevated levels of AM were associated with marked hemodynamic effects, presumably reflecting in part the degree of vasodilatation. We demonstrated significant falls in BP during both low- and high-dose AM infusion. Systolic and

**Urinary Effects of Adrenomedullin Infusion Versus Vehicle Control**

<table>
<thead>
<tr>
<th>Urine Variable</th>
<th>Preinfusion Phase (8:00 to 9:30 AM)</th>
<th>Low-Dose Infusion Phase (9:30 to 11:30 AM)</th>
<th>High-Dose Infusion Phase (11:30 to 1:30 PM)</th>
<th>Postinfusion Phase (1:30 to 3:00 PM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AM Control</td>
<td>AM Control</td>
<td>AM Control</td>
<td>AM Control</td>
</tr>
<tr>
<td>Urine volume, mL/min</td>
<td>4.5 ± 0.7</td>
<td>6.5 ± 1.0</td>
<td>7.2 ± 0.9</td>
<td>8.3 ± 0.7</td>
</tr>
<tr>
<td>Urine Na⁺, mmol/min</td>
<td>0.11 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Urine K⁺, mmol/min</td>
<td>0.07 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.02</td>
</tr>
</tbody>
</table>
diastolic BP fell below time-matched placebo levels after only 1 hour of low-dose infusion at a time when AM levels were within the low pathophysiological range. The peak fall in both systolic and diastolic BP was close to 20 mm Hg and occurred late in the high-dose infusion phase when plasma levels of both AM and cAMP were at their peak. Notably, however, plasma cAMP levels did not rise until the high-dose AM infusion phase, well after the hemodynamic changes were established. The fall in arterial BP exceeded that seen in identical studies of normal volunteers. The incremental rise in plasma AM was almost identical in both studies, as was the effect on CO and HR; however, the mean fall in BP for subjects in the HT group was greater both in absolute terms and in change as a percent of starting BP. This contrasts with the spontaneously hypertensive rat, which exhibited a greater absolute fall in BP with AM injection than normotensive rats but a similar percent change. When data from the 8 normotensives and 8 hypertensives were combined, the peak fall in BP was related to the mean of starting and finishing diastolic and systolic BP, indicating that the greater falls in BP were related to the mean of starting and finishing BP. However, the mean fall in BP for hypertensive subjects in the HT group was greater both in absolute terms and in change as a percent of starting BP. This contrasts with the spontaneously hypertensive rat, which exhibited a greater absolute fall in BP with AM injection than normotensive rats but a similar percent change. When data from the 8 normotensives and 8 hypertensives were combined, the peak fall in BP was related to the mean of starting and finishing diastolic and systolic BP, indicating that the greater falls in BP with increasing BP due to AM may be due to more than just a higher starting BP.

Acknowledgments

We are grateful to the Health Research Council of New Zealand and the National Heart Foundation of New Zealand for financial assistance.

References


Hemodynamic, Hormone, and Urinary Effects of Adrenomedullin Infusion in Essential Hypertension
Richard W. Troughton, Lynley K. Lewis, Timothy G. Yandle, A. Mark Richards and M. Gary Nicholls

Hypertension. 2000;36:588-593
doi: 10.1161/01.HYP.36.4.588
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/36/4/588

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/