Differing thresholds for modulatory effects of adrenomedullin infusion on haemodynamic and hormone responses to angiotensin II and adrenocorticotrophic hormone in healthy volunteers

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ABSTRACT

Experimental data indicate that adrenomedullin (AM) interacts at various levels with the renin–angiotensin–aldosterone system and the hypothalamic–pituitary–adrenal axis, but data from humans are scant. We examined the effects of intermediate-dose, short-term AM infusion on angiotensin II- and adrenocorticotrophic hormone (ACTH)-mediated hormone and haemodynamic responses in healthy subjects. Seven normal volunteers (age 18–25 years) completed a placebo-controlled crossover study. Each subject was studied on day 4 of two periods of a low-salt diet (40 mmol of sodium and 80 mmol of potassium daily), receiving incremental infusions of angiotensin II in the morning and ACTH in the afternoon of each study day, on a background infusion of AM (4 pmol [min⁻¹-kg⁻¹]) or vehicle (hemaccel). Achieved plasma AM levels (23 ± 6 pmol/l) and peak angiotensin II levels (160 pmol/l) were similar on the two experimental days. While the pressor action of angiotensin II was attenuated by AM (P < 0.01) and noradrenaline levels rose (P < 0.05), the aldosterone response was unaltered. During ACTH infusion, AM increased heart rate (P < 0.01), plasma adrenaline (P < 0.01) and plasma noradrenaline (P < 0.05), and augmented the cortisol response (P < 0.01), but was without effect on aldosterone levels and blood pressure. We conclude that the threshold for the effects of AM on aldosterone secretion in humans is set higher than for other biological responses to this hormone, namely blood pressure, heart rate, sympathetic activity and cortisol secretion, under these experimental conditions.

INTRODUCTION

Adrenomedullin (AM) is a vasoactive hormone that is found in tissues and organs throughout the body [1,2]. Gene transcription and synthesis are widespread, but are most prominent in vascular smooth muscle cells and endothelial cells [3–6]. The peptide appears to have a role as an autocrine/paracrine regulator of vascular tone [7–10], and may contribute to homoeostatic responses in sepsis [11] and some cardiovascular disorders [12–18]. AM is also present in the central nervous system, where it is thought to modulate salt appetite, thirst and

Key words: ACTH, adrenomedullin, angiotensin II, arterial pressure, catecholamines, cortisol.
Abbreviations: ACTH, adrenocorticotrophic hormone (corticotropin); AM, adrenomedullin; PRA, plasma renin activity.
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sympathetic activity [19]. In some animal models, AM increases the glomerular filtration rate and has a natriuretic action [20,21].

In addition, AM interacts with the renin–angiotensin–aldosterone system [22–29], the sympathetic system and the pituitary–adrenal axis under some circumstances [19], but data in this regard from humans are scarce [30–32]. In vitro studies have demonstrated inhibition of angiotensin II- and potassium-mediated aldosterone secretion. Our own studies in human volunteers have shown activation of renin during high-dose AM infusion (5.8 pmol·min⁻¹·kg⁻¹), while plasma aldosterone was unchanged [18,30,31]. In one recent study, short-term very-low-dose AM infusion was reported to inhibit angiotensin II-mediated aldosterone secretion [32].

To elucidate more clearly interactions between AM and neurohormonal systems, blood pressure and heart rate, we documented hormonal and haemodynamic responses to incremental infusions of angiotensin II and adrenocorticotropic hormone (ACTH; corticotropin), with and without administration of AM, in healthy young men.

METHODS

The protocol was approved by the Canterbury Ethics Committee. Eight healthy male volunteers (18–25 years) were enrolled in this placebo-controlled, crossover design study. Each took a diet with constant sodium (40 mmol/day) and potassium (80 mmol/day) content for 4 days prior to infusion of AM or vehicle. On both experimental days, the volunteers took breakfast at 07.45 hours, completed a 24-h urine collection at 08.00 hours for sodium, potassium and creatinine measurement, and then remained seated in an easy chair during the morning (angiotensin II) protocol until 11.30 hours. Subjects then remained seated in an easy chair during the afternoon (ACTH) protocol until 15.00 hours. Subjects took sodium and potassium supplements at breakfast (50 ml of hemaccel over 80 min), with a stepped infusion of angiotensin II beginning at 09.20 hours (1.9, 3.8 and 7.6 pmol·min⁻¹·kg⁻¹ for 20 min each). The morning protocol began at 09.00 hours, at which time subjects received an infusion of either AM (4 pmol·min⁻¹·kg⁻¹ for 80 min) in hemaccel or vehicle alone (50 ml of hemaccel over 80 min), followed by a stepped infusion of angiotensin II beginning at 09.20 hours (1.9, 3.8 and 7.6 pmol·min⁻¹·kg⁻¹ for 20 min each). The afternoon protocol began at 13.00 hours, at which time subjects again received either AM (4 pmol·min⁻¹·kg⁻¹ for 80 min) in hemaccel or vehicle alone (50 ml of hemaccel over 80 min), with a stepped infusion of ACTH beginning at 13.20 hours (12.5, 25 and 50 i.u./kg for 20 min each). Subjects were blinded as to which infusion (AM or vehicle) was given; four received AM first and four vehicle.

Venous samples were drawn before, during and after each infusion for measurement of AM, plasma renin activity (PRA), and plasma levels of aldosterone, angiotensin II, noradrenaline and adrenaline, cAMP (commercial kit; Biotrak, Amersham, U.K.), brain natriuretic peptide, atrial natriuretic peptide, endothelin and cortisol. All samples from an individual were analysed in a single assay, as described previously [31]. Intra-assay coefficients of variation were all < 9%. Venous samples were also drawn for measurements of plasma sodium and potassium, and for haematocrit determination, before and on completion of the AM/vehicle infusion phases.

On both infusion days, arterial pressure and heart rate were recorded in duplicate at 10–20 min intervals using an automatic sphygmomanometer (Electronics Services Limited). On four occasions (at beginning and end of the morning protocol, and at beginning and end of the afternoon protocol) after venous sampling, the subjects stood to pass urine for measurements of cAMP, aldosterone, sodium, potassium and creatinine.

Human 52-amino-acid AM for infusion and human angiotensin II were purchased from Clinalfa AG (Darmstadt, Germany). ACTH (Synacthen®) was purchased from Novartis.

Results were analysed using two-way ANOVA, with ‘treatment’ and time as repeated measures. Where a significant effect was seen in any phase, individual time points were analysed using an ANOVA method that compared the difference between AM and vehicle control phases in the change from baseline to a given time point. A P value of 0.05 or less was taken to indicate statistical significance. Results are presented as means ± S.E.M.

RESULTS

The protocols were well tolerated and no symptoms were reported. One subject changed occupation mid-study, leading to extreme levels of physical exertion which resulted in marked discrepancies between study days in baseline urine sodium excretion, PRA and plasma levels of aldosterone, cortisol, angiotensin II and catecholamines. His results were not included in the final analysis. Data collected from the remaining seven subjects were complete and are presented below.

Hormone data

Baseline plasma AM levels for the morning (angiotensin II) and afternoon (ACTH) studies were similar on days of AM and vehicle infusion (6±1–7.9±1 pmol/l). Whereas AM levels remained stable throughout the vehicle control limbs of the study, they increased to peak values of 23±6 and 23±5 pmol/l during AM infusion in
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Figure 1 Plasma levels of AM, cAMP, adrenaline and noradrenaline with AM and vehicle control infusions during the morning (angiotensin II infusion) and afternoon (ACTH infusion) protocols

Epinephrine and norepinephrine are adrenaline and noradrenaline respectively. Results are means ± S.E.M. (n = 7). Significance of differences compared with vehicle: *P < 0.05, †P < 0.01; **P < 0.05 for the entire AM infusion phase.

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The levels were intermediate between those achieved during infusion of low and high doses in our earlier studies [18,31]. Plasma levels of the second messenger cAMP were not altered by AM infusion. Plasma adrenaline levels were similar on AM and vehicle infusion days for the morning study, but with ACTH infusion they were higher during AM compared with vehicle administration (Figure 1). Noradrenaline levels, which were stable or declined during control infusions, increased significantly with AM in both studies (Figure 1). Endothelin levels, measured in the morning study only, were similar at baseline for the two experimental days (1.1 ± 0.1 and 1.1 ± 0.1 pmol/l), and were almost identical during angiotensin II administration with or without AM co-administration (1.3 ± 0.1 and 1.3 ± 0.1 pmol/l at com-
Figure 2  Levels of PRA, plasma angiotensin II, aldosterone and cortisol with AM and vehicle control infusions during the morning (angiotensin II infusion) and afternoon (ACTH infusion) protocols

Results are means ± S.E.M. (n = 7). Significance of differences compared with vehicle: *P < 0.05, †P < 0.01.

Angiotensin II levels increased stepwise during its incremental infusion, and, as expected, PRA showed a reciprocal decline. These effects were not altered by concomitant AM infusion (Figure 2). PRA and angiotensin II levels were stable during the afternoon study, and were similar with AM and vehicle control infusion. Plasma aldosterone levels increased briskly with both angiotensin II and ACTH infusion, with no difference between AM and vehicle control days (Figure 2). Slopes of regression lines for log angiotensin II against linear plasma aldosterone were similar with background AM and vehicle control (P = 0.165 for comparison of slopes).

Plasma cortisol levels declined similarly with AM and vehicle control in the morning study. In the afternoon, cortisol levels were lower at baseline in the AM phase (250±22 compared with 305±22 nmol/l; P = 0.014), but rose during ACTH infusion to be significantly higher than in the vehicle control phase (P = 0.038; Figure 2).
Haemodynamics

Heart rate was similar at baseline in both protocols. In the morning, heart rate fell across the infusion phases, but tended to be higher during AM infusion, such that the change from baseline was greater during AM than during vehicle infusion \((P = 0.022)\) (Figure 3). In the afternoon, heart rate tended to fall during vehicle infusion, but rose significantly during AM infusion to be 10 beats/min greater than for the time-matched vehicle at peak \((P < 0.001\) for phase), before falling below vehicle levels in the recovery phase. Systolic and diastolic blood pressures were well matched at baseline in both protocols. During infusion of angiotensin II, systolic and diastolic blood pressure rose significantly (Figure 3), but levels were lower during AM infusion than during vehicle administration \([\text{peak difference } 5 \pm 1 \text{ mmHg } (P = 0.002)\) for systolic blood pressure and \(3 \pm 1 \text{ mmHg } (P = 0.006)\) for diastolic blood pressure]. During the afternoon, systolic and diastolic blood pressure were similar between phases \((P = 0.46\) and \(P = 0.18\) respectively).

Urinary and plasma biochemical data

Urine volume and the excretion of sodium and potassium were matched at baseline and during infusions in both the morning and afternoon phases (results not shown). Plasma sodium and creatinine levels were also matched at baseline, and were no different during AM and vehicle infusions in the two protocols (results not shown).

DISCUSSION

On the basis of data from \textit{in vitro} and animal studies [19,22,23] we hypothesized that AM infusion would diminish the aldosterone response to angiotensin II, but
not that to ACTH, in healthy volunteers. In order to maximize our chances of discerning any inhibitory action of AM, we chose a low-sodium diet, which enhances aldosterone responsiveness to both angiotensin II and ACTH [33–35]. Under these study conditions, the pressor action of angiotensin II is diminished [33]. Based on previous studies [30,31,36], we chose an intermediate dose of AM (4 pmol min⁻¹·kg⁻¹), infused for a duration (80 min) sufficient to ensure that plasma levels would reach well into the pathophysiological range, but not produce major haemodynamic effects or major stimulation of other hormone systems. We have observed previously that AM adheres avidly to infusion apparatus (E. G. Begg, C. Charles, M. G. Nicholls, M. T. Rademaker, T. G. Yandle, L. K. Lewis and C. J. J. G. Bol, unpublished work), and hence attention to infusion dose and duration is necessary. In this regard, the continuing rise in venous AM levels throughout the infusion period (Figure 1) is noteworthy.

In the event, we observed that AM failed to alter aldosterone responses to either angiotensin II or ACTH. We presume, therefore, that AM given at a dose of 4 pmol·min⁻¹·kg⁻¹ for the brief duration of our study does not alter the aldosterone secretory response to two of its major secretagogues, angiotensin II and ACTH. One caveat here is that AM might have altered the plasma clearance rate of aldosterone (most likely by changing hepatic blood flow), in which case any concomitant modification in the sensitivity of the zona glomerulosa to angiotensin II and ACTH could have been obscured. It is more likely that the threshold for the actions of AM on aldosterone secretion was not reached with the AM dose and duration of infusion used. This result is consistent with our earlier studies, in which any interaction of AM with the renin–angiotensin–aldosterone system occurred at higher doses (5.8 pmol·min⁻¹·kg⁻¹) than were used in the present study [18,31].

Our results contrast with data from Petrie et al. [32], who reported that AM attenuated the plasma aldosterone response, but not the arterial pressure response, to angiotensin II. In that study, Petrie et al. [32] infused AM at a lower dose (3 pmol·min⁻¹·kg⁻¹) and for a shorter time period (60 min) into six subjects receiving a diet of uncertain sodium content. If the subjects in that study were taking a diet of extremely low sodium content (e.g. 10 mmol/day), this might explain the different responses in aldosterone and arterial pressure compared with those in the present study, since, as mentioned already, sodium restriction augments the aldosterone secretory response to angiotensin II, but inhibits its pressor potency [33–35]. Another potential explanation is that we observed substantial haemodynamic and neurohormonal responses to AM, which themselves might have modified the plasma clearance (or perhaps production) of aldosterone; such effects were not noted by Petrie et al. [32]. Finally, differences in doses and duration of infusion of both AM and angiotensin II between the two studies might have contributed to the discrepant results.

In contrast with the negative findings regarding aldosterone responsiveness, we did observe other significant effects of AM. AM was associated with a significant increase in plasma noradrenaline during angiotensin II administration compared with the vehicle control. This might reflect lower arterial pressure with AM, or perhaps AM itself stimulated the sympathetic nervous system, as has been shown in animals and in humans [19,31]. Angiotensin II, which can be shown to stimulate sympathetic activity under some experimental circumstances [37], might have augmented the noradrenaline response to AM and/or the baroreceptor input.

During ACTH infusion, AM stimulated plasma adrenaline and noradrenaline levels and heart rate compared with the vehicle control. This again suggests augmentation of sympathetic activity by AM. Furthermore, AM significantly enhanced the cortisol response to the stepwise infusion of ACTH, although a lack of matching in baseline plasma cortisol levels (prior to ACTH infusion) confounds interpretation of these data. We chose a dose of AM to achieve plasma levels well within the range seen in clinical disorders such as acute myocardial infarction [12], heart failure [13], renal failure [38] and sepsis [11]. Doses of angiotensin II and ACTH were designed to span physiological and pathophysiological plasma levels. Our results may not reflect responses to more prolonged infusion of AM, angiotensin II and ACTH under different conditions of dietary electrolyte intake. Future studies might address these variables.

In summary, we have demonstrated that short-term infusion of intermediate-dose AM in healthy volunteers on a low salt intake was without clear effect on the aldosterone response to either angiotensin II or ACTH. In contrast, intermediate-dose AM inhibited the pressor action of angiotensin II, while stimulating sympathetic activity. During ACTH infusion, AM enhanced the cortisol response, and stimulated adrenaline release and heart rate.

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REFERENCES


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