Adrenomedullin: a hypotensive hormone in man

John G. LAINCHBURY, Garth J. S. COOPER*, David H. COY†, Ning-Yi JIANG†, Lynley K. LEWIS, Timothy G. YANDLE, A. Mark RICHARDS and M. Gary NICHOLLS

Department of Cardioendocrinology, Christchurch Hospital, Christchurch, New Zealand, Developmental Biology and Cancer Research Group, School of Biological Sciences, Department of Medicine, School of Medicine, University of Auckland, Auckland, New Zealand, and Department of Medicine, Tulane University School of Medicine, New Orleans, U.S.A.

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1. Adrenomedullin, a recently discovered 52-amino-acid peptide hormone, circulates in plasma at low picomolar levels in man. Animal studies and studies in vitro indicate that it has diverse biological actions, including vasodilatation, natriuresis and diuresis, and positive inotropism as well as anti-proliferative effects. We investigated the bioactivity of two doses of adrenomedullin in healthy human subjects.

2. Human adrenomedullin was given intravenously to eight male subjects at 2 and 8 ng min⁻¹ kg⁻¹, and haemodynamic, hormonal, renal and biochemical responses were recorded in a placebo (vehicle)-controlled, randomized study.

3. Compared with vehicle, adrenomedullin reduced mean arterial pressure (P = 0.05 for duration of infusion, mean difference at end of infusion 7.7 mmHg), systolic arterial pressure (P = 0.04 for duration of infusion, mean difference at end of infusion 10.7 mmHg) and at the lower dose reduced diastolic arterial pressure (P = 0.05 for lower dose, mean difference at end of infusion 6.3 mmHg) in the absence of compensatory responses in sympathetic activity or renin release. Urine volume and electrolyte excretion were unaffected.

4. The threshold for biological activity of adrenomedullin in man is lower for arterial pressure than for renal or hormonal responses, and is evident at plasma concentrations seen in disorders of the circulation. Adrenomedullin may be an important hormone under pathophysiological circumstances.

INTRODUCTION

The 52-amino-acid adrenomedullin (ADM) was isolated from human phaeochromocytoma in 1993 [1]. The gene is situated in a single locus on chromosome 11 [2], its mRNA is expressed widely [3] and immunoreactive ADM is detectable in numerous tissues, including adrenal medulla, heart, brains, lungs, gastrointestinal organs, spleen and thyroid [4]. The origins of ADM in plasma, typically in the lower picomolar range [5], are uncertain, since venous drainage from various organs, including the adrenals, shows no step-up above arterial levels [6]. In that vascular endothelial cells and vascular smooth muscle cells synthesize and secrete adrenomedullin [7, 8], and receptors are present on vascular smooth muscle [9, 10], there is speculation that the peptide may be important both as a paracrine and autocrine factor and as a classical circulating hormone.

Injection of ADM into experimental animals has elicited a number of responses, most consistently a decline in arterial pressure, increased cardiac output and a diuresis and natriuresis [11]. Interpretation of these responses has often been complicated by the use of very high doses of the peptide in anaesthetized animals. We have completed a study [11a] infusing human ADM into conscious sheep at 10 and 100 ng min⁻¹ kg⁻¹ and observed small changes in intracardiac pressures and blood pressure at the lower dose and an increase in cardiac output at the higher dose. Given that the effects may be greater in the species of origin and that this is the first study of ADM infusions in humans we chose two low dose rates (2 and 8 ng min⁻¹ kg⁻¹) of ADM and administered these in a placebo-controlled, cross-over study to healthy subjects.

METHODS

The study protocol was approved by the Southern Regional Health Authority Ethics Committee (Canterbury, New Zealand). Eight healthy male subjects, ages 31–58 years, taking no medications and having given their informed consent, were studied on day 4 of a diet of constant sodium (150 mmol/day) and potassium (80 mmol/day) content, on two occasions at least 2 weeks apart. On both experimental days subjects took breakfast at 07.45 hours, completed a 24 h collection of urine at 08.00 hours for sodium, potassium and creatinine measurements, then
A venous cannula was placed in either forearm, one for infusion of ADM or vehicle, the other for blood sampling. At 10.00 hours, ADM in Haemaccel was infused at 2 ng min$^{-1}$ kg$^{-1}$ for 90 min then at 8 min$^{-1}$ kg$^{-1}$ for a further 90 min. Alternatively, vehicle alone (50 ml of Haemaccel in 180 min) was administered. Subjects were blinded as to which infusion was being given: four received ADM first, and the order was reversed in the other four. Venous samples were drawn before, during and after each infusion for measurements of plasma renin activity [12], adrenaline and noradrenaline [13], aldosterone [14], cortisol [15], atrial (ANP) and brain (BNP) natriuretic peptides [16, 17] and cyclic AMP (Amersham kits). All samples from an individual were analysed in a single assay. Intra-assay coefficients of variation were between 2.2% for ANP and 12.7% for aldosterone. Venous blood was drawn also for measurements of plasma sodium, glucose, potassium and calcium before and at the completion of ADM and vehicle infusions. For measurement of venous immunoreactive ADM, plasma was mixed with an equal volume of buffer containing 0.1% alkali-treated casein, applied to a pre-equilibrated Sep-Pak Vac C18 cartridge, and the ADM was eluted with 80% propan-2-01/0.013 mol/l HCl. The radioimmunoassay utilized human ADM 1–52 (Peninsula Laboratories, Belmont, CA, U.S.A.) as standard and for iodination, and an antibody, raised locally in New Zealand White rabbits, which was specific for ADM 1–52 having negligible cross-reactivity to ADM 1–12, ADM 13–52 or other vasoreactive peptides. With this assay, the detection limit for ADM was 0.5 pmol/l, the IC$50$ was 1.2 fmol/tube, the intra-assay coefficient of variation was <5%, and recovery of unlabelled ADM 1–52 spiked into plasma was 56%. Results were not corrected for recovery.

On ADM and vehicle infusion days, arterial pressure and heart rate were recorded in duplicate at 15 min intervals using an automatic sphygmomanometer (Electronic Services Ltd). Every 30 min, after venous sampling, the subjects passed urine for measurements of sodium, potassium and creatinine.

Human 52-amino-acid ADM for infusion in man was synthesized on methyl-benzhydrylamine resin using standard solid-phase procedures and was cleaved with hydrogen fluoride/anisole. Sequences containing a disulphide bridge were cyclized by titration with I$_2$ in 90% acetic acid/water solutions [18]. Crude material was purified by gel filtration on Sephadex columns in 50% (v/v) acetic acid followed by gradient elution on C$_{18}$ silica. Homogeneity of the final peptide was assessed by TLC, analytical HPLC, amino acid analysis and matrix-assisted laser-desorption-ionization mass spectrometry. Purity was greater than 98%. The peptide was dissolved in sterile water and diluted with saline giving a concentration of 45 μg/ml (1 μg = 166 pmol of ADM 1–52). Portions of 1 ml were filter-sterilized, dispensed in 1 ml aliquots, freeze-dried and stored at −20°C in sealed ampoules. The vials were tested for sterility and dose after the addition of 1 ml of water for infusion.

Results were analysed using two-way analysis of variance for repeated measures using program P2V of the Biomed Data Processing (BMDP) statistics package [19], with ADM or vehicle administration and time as factors. A P value of 0.05 or less was taken to indicate statistical significance. Values are given as means ± SEM.

RESULTS

No volunteer reported subjective responses to infusion of the peptide.

Plasma ADM levels were 8.9±1.6 and 7.5±0.7 pmol/l before infusion of ADM and vehicle, respectively. At the completion of the 2 and 8 ng min$^{-1}$ kg$^{-1}$ infusion rates of the peptide, plasma ADM levels were 7.6±0.8 and 11.4±2.1 pmol/l, respectively, compared with time-matched values on the control day of 8.3±1.5 and 7.2±0.7 pmol/l (P = 0.04 for the higher infusion rate versus vehicle control).

Compared with vehicle control, ADM induced a fall in systolic arterial pressure which was significant from the onset of infusion to completion of the study (P = 0.04), the difference on average being 10.7 mm Hg at the end of the higher dose infusion (Fig. 1). The fall in mean arterial pressure was significant during the lower of the two infusion rates (P = 0.04) and over the whole 180 min of ADM administration (P = 0.05). Upon completion of infusion, the average difference in mean arterial pressure between the two study days was 7.8 mmHg. The decline in diastolic readings with ADM was significant (P = 0.05) during the lower of the two infusion rates only, the mean difference between study days being 6.3 mmHg at the end of the infusion period. Heart rate was not altered by ADM (Fig. 1).

ADM was without effect on plasma noradrenaline, plasma renin activity and urine sodium excretion (Fig. 2), and did not alter circulating levels of adrenaline, cortisol, aldosterone, ANP, BNP, cyclic AMP, sodium, potassium calcium or glucose (Table 1). Likewise, urine volume, creatinine and potassium were similar on the two experimental days (data not shown).

DISCUSSION

Our study in eight healthy male subjects, the first full report on the biological effects of ADM in man, indicates that the peptide in low dose can reduce arterial pressure. In that this hypotensive action was established after 90 min of infusion and had disappeared 90 min after its completion, it is likely that...
ADM is a rapidly acting hormone. Earlier studies in animals, often under anaesthesia and using high doses of ADM given as a bolus, likewise point to a prompt onset of action [21] but some, at least, report a sustained hypotensive effect [11, 20–23].

Experiments in vitro and studies in intact animals indicate that ADM is a direct arterial vasodilator [11, 20, 24], and this is currently presumed to be the mechanism underlying its hypotensive action. Additionally, however, ADM appears capable of reducing cardiac output under some experimental circumstances [25], and some, but not all, reports suggest that ADM may inhibit activity of the sympathetic nervous system [22, 26]. Whereas our studies cannot provide definitive information on the mechanisms underlying the fall in arterial pressure, the lack of
compensatory increases in plasma renin activity, catecholamines and heart rate raises the possibility that inhibitory effects of ADM on renin release and on the sympathetic system might contribute to its hypotensive action.

Whatever the mechanism(s) underlying the bioactivity of ADM, our results indicate that the peptide is capable of reducing arterial pressure at low picomolar plasma concentrations in healthy man. Using a sensitive and specific radioimmunoassay, the change in plasma ADM was extremely subtle during infusion, and achieved peak plasma levels well within the range reported for patients with cardiac failure and chronic renal failure in particular, and also in essential hypertension [27–29]. At the lower infusion rate measured plasma levels of ADM were in fact lower than at baseline, although not significantly so; this may be related both to the low dose used and possibly to changes in the clearance of ADM which could be augmented, for example by an increase in cardiac output, at the lower infusion rate. The fact that the effects seen on blood pressure were small, and no convincing dose–response was demonstrated with the two doses chosen, suggests that although our infusions were sufficient to cause a biological effect, both doses chosen were in fact low and further studies with higher doses are required to elucidate the magnitude of blood pressure change that may be induced by ADM, and the dose–response relationship. Alternatively, at the higher infusion rate, subtle counterregulatory mechanisms beyond those measured here may have been activated and modulated any further fall in blood pressure. Overall our data suggest that ADM may have biological importance, perhaps as a classical circulating hormone and/or as a paracrine factor released from endothelial cells to induce relaxation of adjacent vascular smooth-muscle cells. There was a small but non-significant difference in baseline ADM levels between study phases; given the careful randomized, cross-over design of the study, this would appear to be a chance finding.

Animal studies show that high-dose ADM, especially when infused directly into the renal artery, can induce a natriuresis and diuresis, and sometimes a kaliuresis [11, 24, 30, 31]. The fact that there were no urinary effects of ADM in our healthy subjects suggests that the dose of peptide was insufficient to reach a threshold for renal bioactivity, or the fall in renal artery perfusion pressure served to negate such bioactivity. Either way, the threshold for biological activity of ADM in healthy man is lower for a change in arterial pressure than it is for enhanced urinary electrolyte excretion.

There is evidence that the biological effects of ADM are mediated by cyclic AMP [11], nitric oxide [24] and decreased intracellular calcium [32]. We observed no rise in plasma cyclic AMP with ADM administration in man, which might be taken to indicate that the hypotensive effect of the peptide was due to non-cyclic AMP mechanisms. Equally, however, ADM at the low doses we employed, may have stimulated cyclic AMP sufficiently in vascular smooth muscle to induce vasodilatation but insufficiently to induce a measurable increase in circulating cyclic AMP. Clearly, additional information is needed in this regard.

Under experimental circumstances, ADM has been shown to inhibit adrenocorticotropin [33, 34] and thence cortisol secretion [34], and to suppress aldosterone production during stimulation with angiotensin II [35, 36]. Although the extremely low doses of ADM used in our protocol were without effect on plasma cortisol and aldosterone levels in man, specifically designed studies are needed to determine whether the peptide is capable of interacting with the pituitary–adrenomedullary and the renin–angiotensin–aldosterone system in man.

In that ADM appears to belong to the calcitonin gene-related peptide superfamily [37], members of which alter calcium or glucose homeostasis, we measured plasma calcium and glucose levels in our study in man. No changes were noted, but again, this does not rule out a modulatory role for ADM under other circumstances.

In conclusion, we have shown that intravenous

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Table 1. Plasma hormone and biochemical indices. Values are means ± SEM. There were no significant differences between phases for the variables measured.

<table>
<thead>
<tr>
<th></th>
<th>Before infusion</th>
<th>2 ng min⁻¹ kg⁻¹</th>
<th>8 ng min⁻¹ kg⁻¹</th>
<th>After infusion</th>
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<tr>
<td>Adrenaline (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>179 ± 20</td>
<td>188 ± 23</td>
<td>191 ± 42</td>
<td>183 ± 36</td>
</tr>
<tr>
<td>Vehicle</td>
<td>180 ± 36</td>
<td>232 ± 54</td>
<td>177 ± 31</td>
<td>193 ± 27</td>
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<tr>
<td>Cortisol (nmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>197 ± 26</td>
<td>220 ± 28</td>
<td>169 ± 27</td>
<td>182 ± 11</td>
</tr>
<tr>
<td>Vehicle</td>
<td>190 ± 29</td>
<td>188 ± 28</td>
<td>165 ± 29</td>
<td>185 ± 32</td>
</tr>
<tr>
<td>Aldosterone (pmol/l)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>181 ± 20</td>
<td>167 ± 28</td>
<td>154 ± 20</td>
<td>132 ± 12</td>
</tr>
<tr>
<td>Vehicle</td>
<td>193 ± 27</td>
<td>193 ± 24</td>
<td>145 ± 15</td>
<td>118 ± 11</td>
</tr>
<tr>
<td>ANP (pmol/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADM</td>
<td>8 ± 1</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>7 ± 1</td>
<td>7 ± 0.5</td>
<td>6 ± 0.5</td>
<td>5 ± 0.5</td>
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<tr>
<td>BNP (pmol/l)</td>
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<tr>
<td>ADM</td>
<td>5 ± 0.6</td>
<td>5 ± 0.6</td>
<td>5 ± 0.6</td>
<td>5 ± 0.7</td>
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<tr>
<td>Vehicle</td>
<td>5 ± 0.7</td>
<td>5 ± 0.5</td>
<td>5 ± 0.5</td>
<td>5 ± 0.6</td>
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<tr>
<td>Cyclic AMP (nmol/l)</td>
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<tr>
<td>ADM</td>
<td>16 ± 0.7</td>
<td>17 ± 0.7</td>
<td>18 ± 0.6</td>
<td>17 ± 0.6</td>
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<tr>
<td>Vehicle</td>
<td>17 ± 0.9</td>
<td>17 ± 0.8</td>
<td>18 ± 1.1</td>
<td>17 ± 0.7</td>
</tr>
<tr>
<td>Sodium (mmol/l)</td>
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<tr>
<td>ADM</td>
<td>140 ± 1</td>
<td>139 ± 2</td>
<td>140 ± 1</td>
<td>139 ± 0.5</td>
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<tr>
<td>Vehicle</td>
<td>139 ± 1</td>
<td>138 ± 0.5</td>
<td>138 ± 1</td>
<td>138 ± 1</td>
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<tr>
<td>Potassium (mmol/l)</td>
<td></td>
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<tr>
<td>ADM</td>
<td>4 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>3.7 ± 0.1</td>
<td>3.9 ± 0.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>3.7 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>3.6 ± 0.1</td>
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<tr>
<td>Calcium (mmol/l)</td>
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<tr>
<td>ADM</td>
<td>2.17 ± 0.03</td>
<td>2.15 ± 0.04</td>
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<td></td>
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<tr>
<td>Vehicle</td>
<td>2.13 ± 0.03</td>
<td>2.13 ± 0.04</td>
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<td></td>
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<tr>
<td>Glucose (mmol/l)</td>
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<tr>
<td>ADM</td>
<td>5 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.9 ± 0.3</td>
<td>4.7 ± 0.2</td>
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</table>
human ADM, which barely altered circulating levels of the peptide, reduced arterial pressure without activating compensatory neurohormonal systems. The threshold for biological effects in man is low for arterial pressure; that for renal and hormonal effects remains to be defined. In that ADM, in addition to proliferative and antimigration activity for vascular endothelin production from both vascular smooth muscle and glomerular mesangial cells [40, 41], can suppress mitogenesis in mesangial cells [42] and may have sympatholymphic properties [22], there is good reason to investigate this peptide further in order to elucidate its physiological and pathophysiological role and to determine whether its actions might be the basis for a new therapeutic approach to disorders of the circulation.

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REFERENCES
