Atrial natriuretic peptide: evidence of action as a natriuretic hormone at physiological plasma concentrations in man

J. V. ANDERSON, J. DONCKIER, N. N. PAYNE*, J. BEACHAM†, J. D. H. SLATER* AND S. R. BLOOM

Departments of Medicine and †Chemical Pathology, Royal Postgraduate Medical School, Hammersmith Hospital, London, and *Cobbold Laboratories, Middlesex Hospital Medical School, London

(Received 21 May/28 August 1986; accepted 18 September 1986)

Summary
1. The administration of exogenous atrial natriuretic peptide (ANP) causes a natriuresis and diuresis in man, but this has, to date, only been demonstrated at plasma ANP concentrations within the high pathological or pharmacological ranges. Evidence that ANP acts physiologically requires the demonstration of a natriuretic effect when it is infused to recreate plasma concentrations similar to those observed after physiological stimuli.

2. We infused human α-ANP (1-28) at a calculated rate of 1.2 pmol min⁻¹ kg⁻¹ for 3 h into seven water-loaded normal subjects, achieving plasma ANP concentrations within the upper part of the physiological range. The subjects' resting plasma ANP concentration increased from 3.8 ± 1.5 to 20.9 ± 1.9 pmol/l.

3. The infusion of ANP caused a 60% increase of mean urinary sodium excretion from 111 ± 18 to 182 ± 30 μmol/min (P < 0.001) and a 28% increase of mean water excretion from 10.8 ± 0.8 to 13.8 ± 1.6 ml/min (P < 0.01).

4. The infusion suppressed mean plasma renin activity from 1.55 ± 0.10 to 1.17 ± 0.06 pmol of ANG 1 h⁻¹ ml⁻¹ (P < 0.001). Mean plasma aldosterone concentration (242 ± 16 basally and 215 ± 15 pmol/l at the end of ANP infusion) did not change significantly. Pulse rate and blood pressure were unchanged throughout the study.

5. No significant change in any of the variables mentioned above occurred during the infusion of the vehicle alone on a separate study day.

6. The demonstration that recreation of plasma concentrations of ANP within the physiological range by intravenous infusion induces a natriuresis provides new evidence supporting the role of ANP as a natriuretic hormone.

Key words: aldosterone, atrial natriuretic peptide, blood pressure, glomerular filtration rate, natriuresis, natriuretic factor, renin-angiotensin system.

Abbreviations: ANG I, angiotensin I; ANP, atrial natriuretic peptide.

Introduction
The discovery that a peptide manufactured by cardiac atrial cells (atrial natriuretic peptide, ANP) can cause natriuretic and diuretic effects in vivo [1] was followed by the demonstration that extracellular fluid volume expansion could release this peptide from the heart into the bloodstream in experimental animals [2, 3] and in man [4, 5]. There has since been intense speculation that a hitherto unrecognized circulating hormone has been discovered [6-8].

Evidence that any substance is a physiologically important circulating hormone is provided in two parts. Firstly, the substance must be released into the bloodstream in response to appropriate stimuli. Secondly, intravenous infusion of a sufficient quantity of the substance to recreate plasma concentrations similar to those seen after physiological stimulation should reproduce the end-organ response. In the case of ANP evidence of release has been provided by a number of workers [2-5, 9-13]. However, although the administration of...
doses of synthetic ANP to experimental animals [14, 15] and man [16, 17] produces a diuresis and natriuresis, these renal effects have only been observed at pharmacological or pathological plasma concentrations. The question of whether physiological plasma concentrations of infused synthetic ANP can elicit a renal response remains unanswered. Such information is required to determine whether or not ANP is a physiologically important circulating hormone. We describe the renal and hormonal response to increasing plasma ANP concentrations within the physiological range.

Methods

Seven healthy male volunteers aged 18–27 (mean 23) years (body weight 54–88, mean 68, kg) were studied in the sitting position from 08.30 to 15.30 hours on two occasions at least 1 week apart. All had fasted from 22.00 hours the previous evening and had abstained from alcohol for the preceding 24 h, and from proprietary drugs for the previous week. The subjects were asked to record what they ate in the 48 h before the first study, and to eat similar meals in the 48 h before the second study. All subjects collected urine for the 24 h immediately preceding each study. They were asked to drink 5 ml of tap water/kg body weight at home at 07.30 hours before each study.

At the start of the study the subjects passed urine and were given a further 5 ml of tap water/kg body weight to drink. Every half hour for the next 7 h they passed urine and were given a quantity of tap water to drink equal to the volume of urine they had just passed, except that at the end of the first half-hour period a further fixed volume (5 ml/kg body weight) of water was drunk to stimulate urine flow. An intravenous cannula was inserted into each forearm: one for peptide infusion, the other for blood sampling.

At the beginning of the sixth half-hour period an infusion of either a calculated dose of 1.2 pmol min⁻¹ kg⁻¹ of human α-ANP (1–28) (batch number 49114; Bissendorf Peptide GmbH, Hanover, West Germany) dissolved in 5% D-glucose solution containing 1.2% human serum albumin (Lister Laboratories, Elstree, Herts., U.K.), or the vehicle solution (placebo) alone (in random order), was started and continued for the next 3 h. The infusion volume was 30 ml administered over the 3 h period. Urine collection was continued for a total of 14 half-hour periods.

Blood pressure (measured by a single observer using a standard mercury sphygmomanometer) and heart rate (measured by counting the radial pulse over 60 s) were recorded mid-way through each half-hour period. Blood samples were taken for ANP measurement (into 10 ml glass tubes containing 60 mg of potassium edetate and 4000 kallikrein inhibitory units of aprotinin), for renin and aldosterone measurement (into 5 ml glass tubes containing 30 mg of potassium edetate) and for serum electrolyte and creatinine measurement (into plain 3 ml polypropylene tubes) at identical time points during both active and placebo studies. A sample of the infusate was taken from the venous end of the infusion cannula immediately after the completion of each infusion and frozen at −20°C. The experimental protocol was approved by the Ethics Committee of the Royal Postgraduate Medical School. All subjects gave written informed consent before taking part in the study.

Blood samples for peptide and steroid measurement were stored on ice and centrifuged within 10 min of collection. The plasma was frozen on solid carbon dioxide and stored at −20°C until the time of assay. Plasma ANP concentrations were measured with a sensitive, specific radioimmunoassay developed at the Royal Postgraduate Medical School [17, 18] after plasma extraction on Sep-Pak C-18 cartridges (Waters Associates) as previously described [19, 20]. All samples were analysed in a single assay with an intra-assay variation of 8%. Plasma renin activity was measured by the method of Menard & Catt [21]. Plasma aldosterone concentration was measured with a Sorin radioimmunoassay kit (CIS, Ballards Lane, London N.12) after solvent extraction of plasma [22]. Serum electrolyte concentrations and serum creatinine concentrations were measured with a Technicon RA-1000 automated analyser.

Data are presented as means ± SEM. The statistical significance of the response of a variable to active or placebo infusion with respect to time, and the significance of the differences in response between the active and placebo studies, were determined by two-way analysis of variance with the Minitab and GLIM programs on the Royal Postgraduate Medical School Computer Centre Perkin Elmer PE 3220 computer. Student's paired t-test was used to compare the 24 h urinary electrolyte excretion of the subjects.

Results

No symptoms were experienced by the volunteers during either the active or the placebo infusions. The mean sodium excretion during the 24 h preceding each study was 189 ± 22 mmol/24 h before the active infusion and 153 ± 21 mmol/24 h before the placebo infusion. This difference did not reach statistical significance.

The mean plasma ANP concentration did not change significantly with respect to time on the day
when the placebo infusion was given. In response to the active infusion the mean plasma ANP concentration rose from 3.8 ± 1.5 pmol/l to 20.9 ± 1.9 pmol/l ($P < 0.001$) and had returned to basal values 25 min after the end of the infusion (Fig. 1).

Urinary sodium excretion and the fractional excretion of sodium did not change significantly during the placebo study. In comparison with this response the infusion of human α-ANP (1–28) at a calculated dose of 1.2 pmol min⁻¹ kg⁻¹ was associated with a highly significant increase of sodium excretion ($P < 0.001$) and of fractional sodium excretion ($P < 0.001$) (Fig. 2). Both sodium excretion and the fractional excretion of sodium changed little during the first hour of infusion, but thereafter showed a progressive increase. During the last half hour of infusion the mean sodium excretion had increased by 60% and the mean fractional excretion of sodium by 48% (Fig. 2). Both parameters remained considerably elevated above pre-infusion values during the hour after the end of the infusion (Fig. 2), despite the plasma ANP concentration having returned to initial values within 25 min.

Water excretion showed a similar response to that of sodium with (in comparison with the placebo study) a highly significant diuresis ($P < 0.005$) (Fig. 2). Phosphate excretion also rose significantly ($P < 0.001$) during the active infusion of ANP in comparison with the response during the placebo study (Table 1). Calcium excretion increased significantly with respect to time during both the active and placebo infusions ($P < 0.001$ on both days) (Table 1). The difference between the responses in the active and placebo studies did not reach significance. There was no significant change in creatinine clearance (calculated for each 30 min urine collection period) on either study day (Table 1). There was no significant correlation between the maximum increment of plasma ANP concentration occurring as a result of infusion of the active agent with the maximum increment of urine sodium excretion ($r = 0.495$, $n = 7$, NS). Similarly, there was no significant correlation between the subjects' urinary sodium excretion during the 24 h before the study and the maximum increment of urine sodium excretion observed in response to ANP infusion ($r = 0.498$, $n = 7$, NS).

Plasma renin activity was unchanged with respect to time during the placebo infusion. In contrast, during active infusion of ANP there was a significant ($P < 0.001$) suppression of plasma renin activity (both with respect to time and in comparison with the placebo study) which increased again towards initial values during the hour after the end of the infusion (Fig. 1). Plasma aldosterone concentrations did not change significantly in response to the ANP infusion (Fig. 1).

There were no statistically significant changes of either pulse rate or blood pressure during the active or placebo studies (Table 1). Radioimmunoassay of the infusate samples revealed that after losses due to adsorption to syringes and tubing the actual dose of ANP infused was 75.5 ± 2.9% of the calculated dose.
Discussion

We have previously established a range of plasma ANP concentration of 1–11 pmol/l in 30 fasting healthy subjects in the erect position at 09.00 hours [23]. Using the same radioimmunoassay it has been shown that the intravenous infusion of normal saline (a stimulus known to induce a natriuresis [24]) raises plasma ANP concentration from a mean of 5.2±0.7 (range 2.0–7.7) pmol/l basally, to a mean peak value of 18.3±5.8 (range 9.9–37.3) pmol/l (n = 7) [4]. The increase of plasma ANP concentration in response to saline infusion in normal subjects has been reported by other groups [5, 25] using different radioimmunoassays. Exertion on a bicycle ergometer can increase plasma ANP concentrations from 5.8±1.4 (range 1.8–10.1) pmol/l basally to 20.3±6.5 (range 8.4–50.7) pmol/l (n = 6) [13]. These physiological changes of plasma ANP concentration contrast with the far higher values seen in pathological states. It has been shown, again using the same assay as in the present study, that chronic renal failure is associated with plasma ANP concentrations of up to 352 pmol/l (n = 20) [20] and supraventricular tachycardia with concentrations of up to 140 pmol/l [26]. In congestive cardiac failure plasma ANP concentrations are markedly elevated before treatment and approach 100 pmol/l [27]. The rise of plasma ANP concentration from 3.8±1.2 pmol/l to 20.9±1.9 pmol/l in response to the infusion of the synthetic peptide in this study therefore causes a change of similar magnitude to that occurring after physiological stimuli in normal subjects. These concentrations are considerably lower than those observed in pathological states, and are far lower than those achieved in any of the reported studies of synthetic ANP infusion in man [17, 28].

The present study therefore demonstrates that recreation of plasma ANP concentrations within the range seen after physiological stimulation, by exogenous administration of the synthetic peptide, causes a natriuresis and diuresis in normal man. This new evidence provides strong support for the hypothesis that ANP is a circulating hormone.

The natriuretic effect of the low dose infusion of ANP used in the present study was delayed, and sodium excretion increased throughout the infusion. Indeed this rise continued in the first 30 min period after the infusion had been stopped, even though plasma ANP concentrations were then falling towards basal values. This lag of biological effect behind changes of plasma concentration has not previously been described and undoubtedly explains why when ANP was infused at a similar low dose for only 30 min in a previous study [17], no significant renal response occurred. Other studies involving the administration of relatively
TABLE 1. Response to the infusion of ANP

Mean ± SEM response of systolic and diastolic blood pressure, pulse rate (measured mid-way through each urine collection period), creatinine clearance, urinary potassium, calcium and phosphate excretion in the two half-hour experimental periods (5 and 6) before, the six half-hour periods during (7-12) and the two half-hour periods after (13 and 14) the intravenous infusion of either a calculated dose of 1.2 pmol min⁻¹ kg⁻¹ of human α-ANP (1-28) (A) or the vehicle alone (control infusion, C) in seven normal water-loaded subjects.

<table>
<thead>
<tr>
<th></th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>112±5</td>
<td>111±5</td>
<td>109±5</td>
<td>107±5</td>
<td>106±4</td>
<td>107±4</td>
<td>105±4</td>
<td>107±4</td>
<td>107±4</td>
<td>108±3</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>72±4</td>
<td>71±3</td>
<td>72±4</td>
<td>72±4</td>
<td>69±2</td>
<td>70±4</td>
<td>71±3</td>
<td>71±3</td>
<td>70±4</td>
<td>72±3</td>
</tr>
<tr>
<td>Pulse rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>60.0±2.3</td>
<td>59.4±1.3</td>
<td>60.3±1.7</td>
<td>57.7±1.2</td>
<td>63.4±1.7</td>
<td>59.4±1.8</td>
<td>60.6±1.5</td>
<td>58.9±1.9</td>
<td>60.6±1.9</td>
<td>60.6±2.3</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>112±7</td>
<td>113±5</td>
<td>123±15</td>
<td>124±8</td>
<td>124±8</td>
<td>124±7</td>
<td>121±8</td>
<td>122±5</td>
<td>122±6</td>
<td>127±7</td>
</tr>
<tr>
<td>Urine potassium excretion (µmol/min)</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>59±8</td>
<td>61±9</td>
<td>63±8</td>
<td>67±10</td>
<td>66±14</td>
<td>63±12</td>
<td>57±11</td>
<td>63±15</td>
<td>67±16</td>
<td>64±13</td>
</tr>
<tr>
<td>Urine calcium excretion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(µmol/min)</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>4.5±1.0</td>
<td>4.0±0.7</td>
<td>4.5±1.0</td>
<td>4.6±1.0</td>
<td>4.8±0.9</td>
<td>5.6±1.0</td>
<td>6.3±1.1</td>
<td>6.7±1.0</td>
<td>6.9±0.9</td>
<td>7.0±0.9</td>
</tr>
<tr>
<td>Urine phosphate excretion (µmol/min)</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>6.5±1.4</td>
<td>8.0±2.4</td>
<td>6.5±1.4</td>
<td>7.7±2.4</td>
<td>7.5±1.7</td>
<td>6.9±2.3</td>
<td>8.4±1.9</td>
<td>7.0±2.0</td>
<td>7.1±2.3</td>
<td>7.4±2.1</td>
</tr>
</tbody>
</table>
massive doses of ANP to animals or to the isolated kidney have been described as showing ANP to have a 'transient duration of action' and that '...the filtration fraction shows a sustained elevation throughout the peptide infusion and diminishes abruptly when the infusion is stopped' [29]. This has led some people to suppose that release of ANP from the heart may be an important emergency regulatory mechanism, but that it may not be functional in normal circumstances. We suggest that the present study demonstrates that ANP does indeed function as a natriuretic hormone under physiological conditions. Under day-to-day conditions a rapid end organ response to a natriuretic hormone would not be required, and indeed it would be disadvantageous if, for instance, changes of plasma ANP concentration due to changes of posture or brief periods of exertion were to change renal sodium excretion immediately. The gradual end organ response to sustained small changes of plasma ANP concentration demonstrated in this study is therefore not unexpected. We postulate that ANP may mediate a natriuretic response to both 'emergency' and physiological stimuli, and that the end organ response differs appropriately under these two sets of conditions.

The infusion of sufficient synthetic ANP to create circulating concentrations in the upper part of the pathological range has been shown to produce suppression of plasma renin activity in man [30] and a similar suppression has been observed in experimental animals [31]. In the present study there were small differences in basal values of plasma renin activity on the active and placebo treatment days which were probably attributable to the small differences in the subjects' sodium intake. Nevertheless the study demonstrated that suppression of plasma renin activity (Fig. 1) occurs at physiological plasma ANP concentrations. Studies in vitro suggest that this suppression of plasma renin activity is due to a direct inhibitory effect of ANP on the juxtaglomerular cells [32]. One recent study of the effects of ANP infusion in man [33] reported a rise of plasma renin activity, in contrast with the suppression seen in both the present study and our previous higher dose study [30]. This apparent discrepancy probably results from the very high dose of ANP used in the former study which resulted in significant hypotension. It seems likely that renin release secondary to this fall of blood pressure overcame the suppressive effect which we have found in the present and previous studies.

As well as suppression of plasma renin activity, there is at least one other point of interaction between the salt-conserving renin-angiotensin-aldosterone system and this putative natriuretic endocrine system, since ANP has already been shown to inhibit angiotensin II-stimulated aldosterone release in man [19]. This latter response would appear to result from a direct effect of ANP on specific receptors [34] in adrenal zona glomerulosa cells [35, 36]. In the present study there was a hint (Fig. 2) that plasma aldosterone concentrations were suppressed during ANP infusion and not during the placebo infusion. The changes were not statistically significant. It is possible that the statistical power of this study of seven subjects was not sufficiently great to detect a small but real suppression of aldosterone secretion.

The site of action of ANP within the kidney remains obscure. Several studies have shown a tendency for creatinine clearance to rise during the infusion of doses of ANP which produce plasma concentrations within the pathological or pharmacological ranges [14, 16, 17, 37]. In the present study no significant change of creatinine clearance occurred during the active infusion. Creatinine clearance is, however, an imprecise index of glomerular filtration rate. Small changes of the glomerular filtration rate could therefore have been responsible for the observed renal response to ANP. Equally specific effects on the renal tubules, or a combination of both glomerular and tubular mechanisms, may be involved. However, the existence of a very high density of specific ANP receptors in the renal glomeruli [38] does suggest that a glomerular mechanism is involved, at least in part, in the renal response.

Specific receptors for ANP are found in abundance in vascular tissue [39]. It was therefore not surprising that studies using very large doses of ANP demonstrated a hypotensive effect in both experimental animals [40] and man [28, 41]. More controlled infusion studies have, however, failed to demonstrate a short-term hypotensive response in normal subjects at plasma ANP concentrations even within the pathological range [17, 37]. The present study confirms, in conscious normal subjects, the absence of a short-term depressor effect at plasma ANP concentrations within the physiological range. What then is the relevance of the specific ANP vascular receptors? It may be that ANP has a very gradual effect on the vascular system which was not revealed by the relatively short (3 h) duration of the infusion in this study. Alternatively, the role of ANP may be to modulate the action of vasoconstrictor agents, rather than it having a direct and wholly independent effect. This hypothesis is supported by experiments in vitro showing a vasorelaxant effect of ANP in vascular strips pre-contracted with vasoconstrictor agents, and little effect on vascular tissue alone [42, 43]. Certainly, in conscious normal subjects the infusion of a dose of ANP which does not change blood pressure...
alone [17, 37], is able to blunt the pressor response to angiotensin II [19].

MacGregor and his colleagues have previously demonstrated physiological plasma release of ANP in response to saline infusion. In the paper discussing their results [5] they state that 'to establish that the atrial peptides control sodium excretion it will have to be shown that infusion of the atrial natriuretic peptides induces sodium excretion at similar plasma levels as those found...following saline infusion'. We believe that the study reported here provides evidence supporting such a physiological role.

Acknowledgments

J.V.A. is supported by a British Heart Foundation Junior Research Fellowship. We thank Mr V. Aber for his invaluable assistance with the computer data analysis.

References


