The plasma release of atrial natriuretic peptide in man

J. V. ANDERSON, J. DONCKIER, W. J. McKENNA AND S. R. BLOOM
Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London

(Received 31 October 1985/17 January 1986; accepted 20 February 1986)

Summary

1. We studied plasma atrial natriuretic peptide (ANP) concentrations in seven normal subjects after the acute intravenous infusion of sodium chloride/potassium chloride solution (saline).

2. Three separate infusions of 6, 12 and 18 ml of saline/kg body weight each significantly increased the circulating concentration of ANP without changes of plasma osmolality or electrolyte concentrations.

3. The mean maximal rise of the plasma ANP concentration after the three saline infusions was significantly correlated \((r = 0.74, P < 0.001)\) with, but occurred 10–30 min later than, the maximal atrial pressure rise.

4. These observations are in accord with the hypotheses that: (a) ANP is a circulating natriuretic factor; (b) atrial distension is an important stimulus to ANP release in man.

Key words: atrial natriuretic peptide, atrial pressure, natriuretic factor, saline infusion.

Abbreviation: ANP, atrial natriuretic peptide.

Introduction

A high concentration of peptides with potent natriuretic activity has been found within human [1] and other mammalian [2, 3] cardiac atria. It has been proposed that ‘atrial natriuretic peptide’ (ANP) is released from the heart in response to atrial distension caused by blood or extracellular fluid expansion and that it exerts homoeostatic control of this volume expansion by the induction of a natriuresis [4, 5]. It has already been demonstrated that ANP is natriuretic in experimental animals and in human subjects [6, 7].

There is good evidence that ANP is released into the blood stream in rats in response to blood volume expansion [8, 9], but this has not yet been demonstrated in man.

We have studied the effect of the intravenous infusion of saline on plasma ANP concentrations and right atrial pressure in normal volunteers in order to determine whether induced increases in atrial pressure are associated with the release of ANP into plasma in man.

Methods

Seven normal male subjects aged 18–37 years (mean \(\pm \text{SEM}\) body weight 72 ± 5 kg) were studied supine at 09.00 hours after an overnight fast. No abnormalities had been determined by clinical history, examination and by routine biochemical screening tests. All subjects had been eating unrestricted diets but denied the use of prescribed or proprietary drugs during the preceding week. A short 1.2 mm diameter plastic cannula was inserted into the left basilic vein. A radiopaque Cordis no. 5 French flush aortic cannula was introduced via the right basilic vein using the Seldinger technique. It was then slid up into the mid right atrium, its position being confirmed by brief (approximately 2 s) radiographic screening with an image intensifier. Right atrial pressure was measured with an Elcomatic (EM 751A) strain gauge pressure transducer and an Electronics for Medicine (VR 12) amplifier (V2203) and display system.

The subjects lay quietly for 30 min. An infusion of a solution of sodium chloride (150 mmol/l) and potassium chloride (3.5 mmol/l) in sterile water (saline) at 37°C was then given through the left venous cannula. A volume of 6 ml/kg was given at a
rate of 250 ml/min. A pneumatic pressure bag was used to compress the flexible intravenous infusion bag to obtain the necessary infusion rate. Two further acute saline infusions (of 12 ml/kg and 18 ml/kg) were given at the same rate after periods of rest lasting 30 min from the end of the previous infusion.

The electrocardiogram was recorded continuously. Blood pressure was measured 5 and 30 min after each saline challenge with a standard mercury sphygmomanometer. Blood was taken from the left venous cannula for ANP measurement into a 10 ml glass tube containing 60 mg of EDTA (potassium salt) and 4000 kallikrein inhibitory units of aprotinin (Trasylol, Bayer Pharmaceuticals Ltd) on two occasions separated by 10 min before the first infusion and at 1, 5, 10, 20 and 30 min after each infusion. In addition, blood samples for plasma electrolytes, urea, creatinine, protein concentration and for osmolality were taken into 5 ml plastic tubes containing 50 units of lithium heparin before and at 5 and 30 min after each infusion.

All subjects gave written informed consent prior to the study. The experimental protocol was approved by the Ethics Committee of the Royal Postgraduate Medical School.

Blood samples were stored on ice and centrifuged for 10 min at 4°C within 10 min of collection. The plasma was frozen on solid carbon dioxide and stored at −20°C until the time of assay. Plasma osmolality was measured by the freezing-point depression method using an Advanced Instruments Inc. Digimatic osmometer model 3D 11. Plasma electrolytes, creatinine, ura and plasma protein concentrations were measured using a Technicon RA-1000 automated analyser.

Atrial natriuretic peptide immunoreactivity in plasma was measured by radioimmunoassay after plasma extraction. Three ml aliquots of plasma were added to 2 g of guanidine hydrochloride (Sigma Chemical Co.) and then were passed through a Sep-Pak C-18 cartridge (Waters Associates) followed by 10 ml of water containing 0.05% (v/v) trifluoroacetic acid. The retained peptides were then eluted from each cartridge into a polyethylene tube with 2 ml of 60% (v/v) acetonitrile in water containing 0.05% (v/v) trifluoroacetic acid. The eluates were evaporated to dryness in a vacuum centrifuge and the resultant pellet reconstituted in 1.2 ml of 60 mmol/l sodium potassium hydrogen phosphate buffer (pH 7.4) containing 0.3% (w/v) bovine serum albumin and 10 mmol/l EDTA (assay buffer). The mean (± sem) percentage extraction of standard quantities of exogenous α-ANP (1–28) added to plasma samples was 79 ± 2% (n = 10). No correction for extraction loss has been made to the data presented.

The antiserum (final dilution of 1:13 000) was raised in rabbits immunized with synthetic human α-ANP (1–28) (Peninsula Laboratories, Merseyside) conjugated to bovine serum albumin with glutaraldehyde. Synthetic human α-ANP (1–28) was radiolabelled with Na125I (Amersham IMS.30, Amersham International plc, Amersham, Bucks, U.K.) by the chloramine T method [10]. The tracer was purified from the iodination reaction products by reverse phase high pressure liquid chromatography. Paired polystyrene tubes containing 400 µl of reconstituted plasma extract were assayed in duplicate (total volume 800 µl). The synthetic peptide was used as standard. After incubation at 4°C for 4 days the bound radiolabel was separated from the free radiolabel by the addition of 4 mg of dextran-coated charcoal/tube, followed by centrifugation at 2500 g for 30 min. The assay could detect 1 fmol of ANP immunoreactivity/tube with 95% confidence. All samples were included in a single assay (intra-assay variation 9%).

Data are presented as means ± sem. The statistical significance of the change of the ANP concentration in response to the saline infusion was determined by analysis of variance using the MINITAB program on the Royal Postgraduate Medical School Computer Centre Perkin-Elmer PE 3200 computer. All other statistical comparisons were performed using Student’s t-test.

Results

None of the subjects experienced any side effects during the study. A sense of fullness in the neck and face was noticed by all the volunteers during and for 1–2 min after the highest but not the lower volume saline challenges. Four of the volunteers experienced some discomfort due to bladder distension with urine during the last 30 min of the study.

The mean basal plasma concentrations of ANP were similar (4.7 ± 0.9 and 5.2 ± 0.7 pmol/l) when measured on the two occasions 10 min apart before the start of the study. The mean right atrial pressure rose significantly and was maximal 1 min after the end of each saline infusion (Fig. 1). After the 6 ml/kg and 12 ml/kg saline challenges the mean right atrial pressure had returned to basal values within 30 min. After the 18 ml/kg saline challenge the 30 min pressure recording was still significantly above basal values. In contrast to the changes of mean right atrial pressure, the plasma concentration of ANP rose significantly (P < 0.005) after each volume challenge, but had not returned to basal values after 30 min (Table 1). For this reason the rise of the plasma ANP concentration above its level immediately before each saline challenge is
Table 1. Response to the acute administration of physiological saline (NaCl/KCl solution) in seven healthy subjects

<table>
<thead>
<tr>
<th>Basal</th>
<th>6 ml/kg saline infusion</th>
<th>18 ml/kg saline infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 min after</td>
<td>30 min after</td>
</tr>
<tr>
<td>Plasma sodium (mmol/l)</td>
<td>143.8 ± 1.1</td>
<td>143.6 ± 0.9</td>
</tr>
<tr>
<td>Plasma potassium (mmol/l)</td>
<td>4.3 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>Plasma urea (mmol/l)</td>
<td>5.2 ± 0.4</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>Plasma creatinine (mmol/l)</td>
<td>82.7 ± 2.7</td>
<td>80.7 ± 2.8</td>
</tr>
<tr>
<td>Total protein (g/l)</td>
<td>73.0 ± 4.7</td>
<td>70.5 ± 4.8</td>
</tr>
<tr>
<td>Plasma ANP (pmol/l)</td>
<td>7.1 ± 1.1</td>
<td>7.2 ± 2.2</td>
</tr>
<tr>
<td>Right atrial pressure (mmHg)</td>
<td>116 ± 6.1</td>
<td>118 ± 5.8</td>
</tr>
<tr>
<td>Plasma ANP concn. (pmol/l)</td>
<td>78 ± 3</td>
<td>78 ± 3</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 ± 3</td>
<td>78 ± 3</td>
</tr>
</tbody>
</table>

*P < 0.05; †P < 0.01.

Plasma release of ANP in man

illustrated in Fig. 1. After the 6 ml/kg saline challenge the incremental plasma ANP concentration rose steadily throughout the 30 min observation period. After the two larger challenges there was a peak increment of plasma ANP concentration after 10 min which then subsided, but was still significantly above initial values at 30 min. There was a significant correlation between the maximum increment of the ANP concentration and the maximum increment of right atrial pressure (r = 0.74, P < 0.001) after the saline challenges.

The plasma concentrations of sodium, potassium and the plasma osmolality remained unchanged throughout the study (Table 1). Both the plasma

![Graph](a)

![Graph](b)

Fig. 1. Mean right atrial pressure (mean ± SEM) (a) and the change of plasma ANP concentration (b) in response to the acute infusion of 6 (-----), 12 (-----) and 18 (-----) ml/kg of isotonic sodium chloride/potassium chloride solution. Time zero represents the end of each infused intravenous saline challenge.
protein and creatinine concentrations fell below baseline values 5 min after the first (6 ml/kg) challenge \((P < 0.01)\) and fell progressively thereafter. Similar but less striking changes occurred in plasma urea concentration (Table 1). The systolic and diastolic blood pressures were unchanged after both the 6 and the 12 ml/kg saline challenges, but were significantly elevated both 5 and 30 min after the 18 ml/kg challenge (Table 1).

Discussion

It is long established that saline infusion increases sodium excretion. The experiments of de Wardener and his colleagues [11, 12] have demonstrated that the natriuresis induced by the intravenous infusion of saline can occur in the absence of changes in renal haemodynamics and aldosterone, thus indicating the presence of a humoral ‘third factor’. If sodium excretion can be mediated by a humoral agent, saline infusion should increase its concentration in plasma. We therefore used saline loading to determine whether it could induce changes of the plasma concentration of the putative natriuretic hormone ANP in man.

It has previously been demonstrated that ANP is detectable in plasma [13], and that the concentration of ANP in blood from the coronary sinus is considerably higher than that in peripheral blood [14]. This finding strongly suggests that the heart is the source of the ANP found in the peripheral circulation.

The observation in this study of the significant correlation between the changes of plasma ANP concentration and the changes of right atrial pressure induced by saline loading are consistent with the hypothesis that, in man, release of ANP in vivo from the heart into plasma occurs in response to atrial distension. This release of ANP occurred without changes of plasma osmolality, or of the plasma sodium or potassium concentrations. The progressive fall in the plasma protein, creatinine and urea concentrations reflects the haemodilution produced by the saline infusions. It is interesting that the smallest saline challenge (6 ml/kg) produced an increase of plasma ANP concentration maximal at least 30 min after the end of the infusion, whereas the larger saline challenges (12 ml/kg and 18 ml/kg) produced peak ANP concentrations 10 min after the end of the infusion. In the light of these observations we speculate that two mechanisms could be responsible for ANP release: an early release of ANP into plasma in response to relatively large extracellular volume changes and a slower more gradual mechanism capable of responding to smaller changes of extracellular volume. The marked increase in the plasma concentrations of ANP (in the order of 500 pmol/l) previously observed in the blood of rats very shortly after large (from 15% to 50%) increments of blood volume produced by the intravenous infusion of colloid [8] may represent release by the more rapid mechanism.

The data in Fig. 1 illustrate that the release of ANP into plasma and the changes of atrial pressure after acute saline challenge were not immediately related in time. The atrial pressure was maximal when first measured 1 min after the end of each saline challenge and swiftly fell towards basal values. In contrast, at the lowest (6 ml/kg) volume of saline infused the plasma ANP concentration increased only gradually and was maximal at 30 min by which time the atrial pressure was identical to basal values. Even the earlier response of the plasma ANP concentration to the larger volumes of saline (12 ml/kg and 18 ml/kg) occurred long after the atrial pressure had begun to return towards initial values. It is possible to speculate that the early rise of atrial pressure after saline infusion distends the atria and initiates a process which produces release of ANP into plasma some minutes later. We have previously found that the first phase elimination half-life of ANP in plasma is short, in the order of 2–3 min [15]. The plasma ANP response must therefore be closely related in time to atrial secretion. There is little evidence to date to suggest whether this release of ANP is produced directly by atrial distension or whether neural reflexes are involved, although the demonstration that ANP can be released from the isolated beating heart by increased filling pressures [9] implies that the former mechanism could be of greater importance.

It is possible that the significant rise of blood pressure occurring after the largest saline load (Table 1) could be attributed to bladder discomfort in addition to any direct effect of the saline loading per se.

The demonstration that saline infusion produces release of ANP into plasma, and that the magnitude of the release is related to changes of atrial pressure, supports the putative role of ANP as a circulating natriuretic hormone in man.

Acknowledgments

J.V.A. is supported by a British Heart Foundation Junior Research Fellowship. We thank the nursing and technical staff of the Hammersmith Hospital cardiac catheterization laboratory for their cheerful support, and A. C. R. Burns for assistance in the radioimmunoassay laboratory.
References


