Effect of atrial natriuretic peptide on blood pressure, guanosine 3'5'-cyclic monophosphate release and blood volume in uraemic patients

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SUMMARY

1. Eleven patients on chronic maintenance dialysis were investigated before and after intravenous bolus injection of atrial natriuretic peptide (2 μg/kg body weight).

2. Mean blood pressure was reduced to the same extent in the uraemic patients as in 11 healthy subjects, with a nadir 3 min after the atrial natriuretic peptide injection at which time mean blood pressure was reduced by 13% (median) in the uraemic patients and 11% in the healthy subjects.

3. Basal plasma atrial natriuretic peptide and guanosine 3':5'-cyclic monophosphate levels were higher in the uraemic patients than in the healthy subjects, but guanosine 3':5'-cyclic monophosphate increased markedly in both groups after atrial natriuretic peptide injection.

4. Using changes in γ-emission from blood after previous labelling of erythrocytes with 51Cr, and changes in packed cell volume, haemoglobin and erythrocyte count, a reversible shift of fluid from the intravascular phase was demonstrated in the uraemic subjects. The blood volume was maximally reduced by 6% (median) of initial blood volume at 30 min after atrial natriuretic peptide injection.

5. Correlation analyses gave no evidence of a causal relationship between the changes in mean blood pressure and changes in blood volume, angiotensin II, aldosterone or arginine vasopressin after atrial natriuretic peptide injection.

6. It is concluded that a pharmacological dose of atrial natriuretic peptide does not seem to be mediated by its diuretic effect or ability to displace fluid from plasma to the interstitial fluid compartment.

Key words: atrial natriuretic peptide, blood pressure, blood volume, guanosine 3':5'-cyclic monophosphate, uraemia.

Abbreviations: ANP, atrial natriuretic peptide; BP, blood pressure; BV, blood volume; CRF, chronic renal failure; cyclic GMP, guanosine 3':5'-cyclic monophosphate; HR, heart rate; PCV, packed cell volume.

INTRODUCTION

The plasma level of atrial natriuretic peptide (ANP) is known to be increased in patients with chronic renal failure (CRF) [1, 2], but it remains to be established whether ANP plays a role in blood pressure (BP) regulation in these patients, and whether the stimulatory effect on guanosine 3':5'-cyclic monophosphate (cyclic GMP) release is still preserved despite the habitual high ANP. ANP has BP-reducing properties [3, 4], but it is as yet unclear to what degree this is mediated through diuretic effects [3, 5], displacement of fluid from plasma to the interstitial fluid compartment [3, 5, 6], vasodilatation [7] and/or reduction of cardiac output [8].

The aims of the present study were: (1) to evaluate the effect of ANP on BP and heart rate (HR) in patients with CRF on maintenance dialysis compared with the response in healthy subjects, (2) to compare the cyclic GMP release after ANP injection in uraemic patients and healthy subjects, (3) to determine whether changes in BP after ANP injection were related to displacement of fluid from plasma to the interstitial fluid compartment in patients with CRF in whom the diuretic effect plays no role, and (4) to discover whether ANP injection had any effect on plasma levels of angiotensin II, aldosterone and arginine vasopressin in the uraemic subjects.
EXPERIMENTAL

Patients

Eleven patients with CRF on maintenance dialysis were studied: eight men and three women with a median age of 38 years (range 23–52 years). All had a 24 h urine volume of less than 300 ml; three of the patients were anephric. The primary kidney diseases were chronic glomerulonephritis (two patients), congenital renal hypoplasia (two patients), chronic interstitial nephritis (one patient) and of unknown aetiology (six patients). None of the patients was on antihypertensive therapy, and there was no clinical or laboratory evidence of heart failure, hepatic failure or diabetes mellitus. Five of the patients were on continuous ambulatory peritoneal dialysis and six of the patients were on haemodialysis. None of the patients had oedema on clinical examination and the patients on haemodialysis were dialysed to dry weight on the day before the investigation.

Eleven healthy subjects were studied: eight men and three women, with a median age of 43 years (range 26–60 years), without any clinical or laboratory evidence of diseases of the heart, kidneys, liver or endocrine organs.

All individuals studied gave their consent to participate in the investigation after having been informed of the nature and the purpose of the study, according to the regulations of the local ethics committee.

Procedure

Studies were performed in the morning after a fasting period of 8 h. The patients stayed in the supine position during the whole investigation. Basal BP was established as the average of two measurements taken in the morning after 30 min in the supine position and two measurements taken just before intravenous injection of ANP (2 μg/kg body weight; Bissendorf Peptides, Wedemark, F.R.G.) during 1 min. BP and HR were measured 1, 3, 5, 7, 9, 15, 30, 60, 90 and 120 min after termination of the ANP injection. Blood volume (BV) was determined before ANP injection and changes in BV were determined 10, 20 and 30 min after injection. Blood for measurement of ANP and cyclic GMP was drawn before, 2, 10 and 30 min after ANP injection and blood for determination of angiotensin II, aldosterone and arginine vasopressin was drawn before and 30 min after ANP injection. Blood was drawn from the arm that was not used for injection of isotopes and ANP.

The BP response and the changes in plasma levels of ANP and cyclic GMP after the same dose of ANP were investigated in the healthy subjects.

Methods

BP and HR were measured with an automatic BP-recording device (Copal, Japan) and a Hawksley random zero sphygmomanometer was used as a control at the beginning and the end of each investigation. Phase V of the Korotkoff sounds was taken as the diastolic BP.

Blood drawn for analysis was replaced by a similar volume of saline (150 mmol/l NaCl).

BV was determined as the sum of plasma volume and erythrocyte volume. Plasma volume was determined after injection of 125I-labelled albumin (5 μCi = 0.2 MBq). Blood samples were drawn 10, 20 and 30 min after injection, and after extrapolation on semilogarithmic graph paper, the radioactivity of the plasma at 0 min was used in the calculation of plasma volume. Erythrocyte volume was determined after labelling of erythrocytes with 51Cr. A 16 ml citrate-glucose-stabilized blood specimen was drawn and, after plasma removal, incubated with 50 μCi (1.9 MBq) of 51Cr at room temperature for 30 min. The erythrocytes were washed twice with saline, resuspended in saline to a packed cell volume (PCV) of about 30, and 10 ml was re-injected (10 μCi = 0.4 MBq). γ-Emission from blood drawn 30 min after the injection and haemolysed through refrigeration was used in the calculation. All total counts minus background were higher than 10000 and 51Cr values were corrected for decay during the counting time. Using the same method, we have previously measured BV per m² surface area (S = 0.007184 × weight²0.425 × height0.725, according to the formula of DuBois) in 21 healthy men with a median age of 41 years (range 26–63 years) and found a median at 2970 ml/m² (range 2540–3500 ml/m²). In 10 healthy women with a median age of 43 years (range 23–63 years) BV per S was 2470 ml/m² (range 2290–3290 ml/m²).

Whole-body PCV was determined as:

\[
\text{erythrocyte volume + plasma volume}
\]

and the f ratio as:

\[
f = \frac{\text{whole-body PCV}}{\text{peripheral PCV}}
\]

The f ratio was 0.87 (0.82–0.92) in the 31 healthy subjects.

BV at time t after ANP injection was determined as:

\[
BV_t(a) = \frac{(BV_0 - p)a_0}{a_1}
\]

where BV0, t is the initial BV, p is the volume of blood taken from time 0 to time t, a0 is the 51Cr γ-emission from a 2 ml blood specimen taken at time 0 and a1 is the 51Cr γ-emission from a 2 ml blood specimen taken at time t. BV/(PCV), BV/(Hb) and BV/(Ery) were determined similarly and BV was calculated as \[BV(a) + BV/(PCV) + BV/(Hb) + BV/(Ery)]/4. PCV was determined by microtube centrifugation. Haemoglobin (Hb) and erythrocyte count (Ery) were measured using a Coulter counter (Centralaboratoriet, Aarhus Kommunehospital).

Blood for measurement of ANP and cyclic GMP was kept on ice and centrifuged within 30 min. Ethylenediaminotetra-acetate was used as anticoagulant and stabilizer. Aprotinin was also added to the ANP tubes. ANP was determined by radioimmunoassay as previously
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ANP was extracted from plasma by means of Sep-Pak C-18 Cartridges (Water Associates) using 80% (v/v) ethanol in 4% (w/v) acetic acid. The recovery was 75% and the coefficients of variation were 12% (interassay) and 10% (intra-assay). Cyclic GMP was measured using a radioimmunoassay kit (Amersham). Ethanol was used for extraction from plasma. The recovery was 90% and the coefficients of variation were 9% (interassay) and 6% (intra-assay).

Angiotensin I was determined by a slight modification of the method described by Kappelgaard et al. [10]. It was measured by radioimmunoassay after previous extraction of plasma with a cation resin and subsequent elution from the resin with methanol and ammonia/methanol. The recovery was 80% and the coefficients of variation were 13% (interassay) and 9% (intra-assay). Aldosterone was measured by a slight modification of the method of Rask-Madsen et al. [11] using a radioimmunological assay on the residue from plasma prepared by extraction with dichloromethane and purification on silica gel columns. The recovery was 90% and the coefficients of variation were 12% (interassay) and 8% (intra-assay). Arginine vasopressin was measured by radioimmunoassay [12] using a modification of the method of Robertson et al. [13]. Radioimmunoassay was performed after precipitation of plasma proteins with cold acetone and extraction of lipids with petroleum ether. The recovery was 95% and the coefficients of variation were 13% (interassay) and 10% (intra-assay). All hormone values were corrected for non-ideal recovery.

Statistical analysis

Non-parametric statistical tests were used for the statistical analysis. Friedman's test and Wilcoxon's signed rank test were used for within-group comparisons and Mann-Whitney's U-test for comparisons between groups. Spearman's test was used for correlation analysis. P values of less than 5% were considered statistically significant. Results are given as median (range).

RESULTS

BP and HR

The changes in mean BP (diastolic BP plus one-third of pulse pressure) and HR after ANP injection in patients with CRF and healthy subjects are given in Fig. 1. The reduction in mean BP reached a nadir 3 min after ANP injection in both groups. No significant difference existed at any time in the reduction of mean BP between patients with CRF and healthy subjects with regard to both absolute and relative changes.

Basal mean BP was significantly lower in the patients with CRF (median 70 mmHg, range 57–92 mmHg) than in the healthy subjects (91 mmHg, 81–109 mmHg; P < 0.01). Basal systolic BP was the same in patients with CRF (median 105 mmHg, range 76–128 mmHg), and in the healthy subjects (119 mmHg, 103–136 mmHg). Basal diastolic BP was significantly lower in the patients with CRF (median 58 mmHg, range 43–77 mmHg) than in the healthy subjects (79 mmHg, 70–95 mmHg; P < 0.01).

HR increased after ANP injection in both groups (Fig. 1). One minute after ANP injection the increase in HR was significantly lower in patients with CRF than in healthy subjects both absolutely (P < 0.02) and relatively (P < 0.02), but on all other occasions after ANP injection no significant difference existed between the two groups. Basal HR was 68 min⁻¹ (range 56–85 min⁻¹) in the patients, which was significantly higher than in the healthy subjects (63 min⁻¹, 52–75 min⁻¹; P < 0.05).

ANP and cyclic GMP

The values for ANP, cyclic GMP and cyclic GMP/ANP in plasma before and after ANP injection are given in Table 1. Basal ANP and ANP after ANP injection were significantly higher in patients with CRF than in healthy subjects.

Cyclic GMP was higher in the uraemic patients than in the healthy subjects, both before and 2, 10 and 30 min after ANP injection. After ANP injection cyclic GMP increased markedly in both groups. The absolute increase in cyclic GMP was higher in the uraemic patients than in the healthy subjects at 2, 10 and 30 min after ANP injection (all P < 0.01). The relative increase from basal values of cyclic GMP was, however, higher in the healthy subjects than in the patients (P < 0.05, P < 0.01 and P < 0.02 at 2, 10 and 30 min after ANP injection, respectively).

The cyclic GMP/ANP ratio was higher in the uraemic patients than in the healthy subjects both before and 2 and 10 min after ANP injection. Thirty minutes after ANP injection the difference did not reach significance (P = 0.07).
Table 1. Plasma concentrations of ANP and cyclic GMP and the cyclic GMP/ANP ratio before and 2, 10 and 30 min after intravenous bolus injection of ANP (2 μg/kg body weight) in 11 patients with CRF on maintenance dialysis and in 11 healthy subjects (control)

Results are shown as median (range). Abbreviation: NS, not significant.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>2 min after</th>
<th>10 min after</th>
<th>30 min after</th>
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<tbody>
<tr>
<td>ANP (pmol/l)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CRF</td>
<td>16.6 (6.5-107)</td>
<td>1271 (459-3616)</td>
<td>117 (81.8-612)</td>
<td>33.4 (14.0-63.5)</td>
</tr>
<tr>
<td>Control</td>
<td>3.4 (2.8-6.5)</td>
<td>713 (158-3087)</td>
<td>85.3 (41.0-201)</td>
<td>10.0 (5.9-15.6)</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
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<tr>
<td>Cyclic GMP (nmol/l)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>32.4 (15.3-97.0)</td>
<td>78.4 (52.0-144)</td>
<td>108 (88.0-209)</td>
<td>123 (72.5-192)</td>
</tr>
<tr>
<td>Control</td>
<td>4.6 (2.5-5.7)</td>
<td>18.4 (7.1-29.7)</td>
<td>32.6 (21.6-42.7)</td>
<td>22.0 (16.0-38.8)</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Cyclic GMP/ANP ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRF</td>
<td>2130 (220-8310)</td>
<td>63 (23-266)</td>
<td>1000 (158-2170)</td>
<td>2920 (1960-6800)</td>
</tr>
<tr>
<td>Control</td>
<td>1330 (440-1540)</td>
<td>24 (5-57)</td>
<td>373 (142-795)</td>
<td>2130 (1210-3060)</td>
</tr>
<tr>
<td><em>P</em></td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

Fig. 2. Relative changes in BV after ANP bolus injection (2 μg/kg body weight) in 11 patients with uraemia. Results are medians and one quartile.

Changes in BV after ANP injection

The changes in BV measured 10, 30, 60, 105 and 150 min after ANP injection are given in Fig. 2, and the individual estimates of BV changes by the different methods are given in Table 2. A reversible significant (*P*<0.01) shift of fluid with a maximum of 6% (median) of initial BV at 30 min after ANP injection was found. A significant negative correlation between changes in BV and changes in mean BP was found 10 min after ANP injection (Fig. 3). At 30 min (*ρ* = -0.116) and 60 min (*ρ* = -0.280) after ANP injection the changes in mean BP and the changes in BV were not significantly correlated.

**BV**

BV per m² surface area was 2680 ml/m² (median) (range 1960-3530 ml/m²) in the patients with CRF. BV per S in patients with CRF divided by the median value for healthy subjects of the same sex was 0.95 (0.74-1.19). BV was not significantly different from values in healthy subjects but the variability was higher in the patients with CRF. BV per S divided by the median value for healthy subjects of the same sex was the same in patients on continuous ambulatory peritoneal dialysis (0.95, 0.91-1.03) as in patients on haemodialysis (0.90, 0.74-1.19). The f ratio was the same in the patients with CRF (median 0.87, range 0.80-0.94) as in the healthy subjects.

**Angiotensin II, aldosterone and arginine vasopressin**

The plasma values of angiotensin II were 9.6 pmol/l (median) (range 2.0-82.2 pmol/l) before and 12.4 pmol/l (2.0-77.4 pmol/l) 30 min after ANP injection. Aldosterone was 72 pmol/l (42-1590 pmol/l) before and 89 pmol/l (42-1510 pmol/l) 30 min after ANP had been given; and arginine vasopressin was 1.8 pmol/l (0.9-3.2 pmol/l) before and 1.9 pmol/l (0.8-4.1 pmol/l) after ANP injection. The ANP injection induced no significant change in these hormones; likewise, no significant change was found when the patients without kidneys in situ were excluded from the analyses. Changes in BP measured 30 min after ANP injection were not correlated to changes in angiotensin II, aldosterone or arginine vasopressin.

**DISCUSSION**

In the present study we found that ANP reduced BP to the same degree in CRF patients on maintenance dialysis...
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Table 2. Relative reduction of BV 10, 30, 60, 105 and 150 min after intravenous administration of ANP (2 μg/kg body weight) to 11 dialysis patients, as calculated from changes in γ-emission from 51Cr in blood specimens or changes in PCV, haemoglobin and erythrocyte count.

Results are shown as medians (first to third quartiles).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Reduction in BV (%)</th>
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<tbody>
<tr>
<td></td>
<td>51Cr method</td>
</tr>
<tr>
<td>10</td>
<td>3.0</td>
</tr>
<tr>
<td>30</td>
<td>5.8</td>
</tr>
<tr>
<td>60</td>
<td>2.4</td>
</tr>
<tr>
<td>105</td>
<td>2.1</td>
</tr>
<tr>
<td>150</td>
<td>0.3</td>
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</table>

whether ANP plays a role in BP regulation in patients on maintenance dialysis in whom the diuretic effect is of no importance. On the one hand, the BP-lowering effect of ANP might be less in patients with chronically elevated endogenous ANP, due to prior occupancy and/or down-regulation of the receptors, but, on the other hand, the BP-lowering effect of ANP may be enhanced due to impaired baroreceptor function [16, 17] and due to a retarded degradation of ANP in CRF [18]. In this study we demonstrated that the BP-depressing effect was exactly the same in patients with CRF and in healthy subjects, although the early counter-regulatory increase in HR was slightly more pronounced in the healthy subjects BP reduction was greatest during the first minutes after injection of ANP, but was still present 2h later in accordance with previous findings [19], suggesting a more prolonged effect of ANP than its presence in plasma might infer.

Plasma levels of ANP and cyclic GMP were higher in uraemic patients than in healthy subjects both before and after ANP injection, but despite this, the absolute increase in cyclic GMP after ANP injection was more pronounced in uraemic patients than in healthy subjects [21, 22]. The relative increase in cyclic GMP, however, was less than in the healthy subjects. As an index of the responsiveness of cyclic GMP production in relation to ANP stimulation, we have introduced the term cyclic GMP/ANP ratio. It seems that not only ANP but also the cyclic GMP/ANP ratio is increased in CRF, and the responsiveness of cyclic GMP production after a pharmacological dose of ANP is preserved despite elevated endogenous concentrations of ANP in CRF. The degradation of cyclic GMP may be slightly prolonged in these patients [23].

The BP-reducing effect of ANP may be mediated through: (1) its diuretic and natriuretic effects [3, 5], (2) displacement of fluid from plasma to the interstitial fluid or the intracellular compartment [3, 5, 6], (3) reduction of vascular resistance [7, 24], and/or (4) a reduction of cardiac output because of a decrease in cardiac filling.
pressure or a direct effect on the myocardium [8]. In this study, changes in BV after ANP administration were determined in patients with CRF in whom interference from the diuretic effects of the peptide plays no role. As previously demonstrated in healthy subjects [3] and in nephrectomized rats [6, 25, 26], ANP induced haemo-concentration, but we also demonstrated that this effect was clearly reversible reaching its maximum 30 min after ANP injection. At 10 min after ANP injection, when BP was still at its nadir, the reduction in BP was strongly negatively correlated to the reduction in BP, and the maximal reduction in BP occurred before the maximal displacement of fluid. Thus, increased plasma levels of ANP may contribute to maintain plasma volume within near-normal limits in CRF [27], but the ability of ANP to displace fluid from plasma does not seem to be causally related to the reduction in BP. ANP may elevate capillary permeability directly [28], the displacement of fluid being more pronounced the higher the intracapillary pressure. Our results suggest some degree of interdependence between arterial BP and capillary pressure because patients with the most profound BP reduction had less displacement of fluid. Intracapillary pressure may further be increased after high-dose ANP bolus injection due to veno-constriction induced by ANP, or induced by baroreflex activation [26].

It has been proposed that ANP has an inhibitory effect on the production of water- and sodium-retaining and BP-elevating hormones like angiotensin II, aldosterone and arginine vasopressin. In the present study the plasma level of these hormones was unchanged 30 min after a high dose of ANP. Similar results were obtained in our laboratory in healthy subjects (H. Eiskjaer et al., unpublished work). It is, however, possible that had it not been for an inhibitory effect of ANP, the hormone levels might have increased due to the reduction in BP and BV [29, 30].

In conclusion, high-dose ANP bolus injection has the same BP-reducing effect in CRF patients on maintenance dialysis as in healthy subjects, and the stimulatory effect on cyclic GMP release seems to be preserved in uraemic patients. The BP-reducing effect of ANP cannot be attributed to the reversible displacement of fluid from plasma shown in the uraemic patients and does not seem to be dependent on its diuretic effect.

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REFERENCES

20. Reference deleted.


