The effects of low dose intravenous 99-126 atrial natriuretic factor infusion in patients with chronic renal failure

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Summary: The aim was to study the renal and hormonal effects of intravenous 99-126 atrial natriuretic factor (ANF) infusion in a mixed group of patients who had moderate to severe chronic renal failure (CRF) and who were not treated with dialysis. The peak mean plasma level of ANF achieved during the experiment was at the upper limit of an absolute range of basal values previously recorded in a larger group of patients with similar degrees of renal impairment. A significant tissue effect was confirmed by rises in plasma and urinary cyclic guanosine monophosphate, the 'second-messenger' of ANF. ANF infusion increased sodium excretion rate by a mean of 68% compared with a fall of 40% in a placebo group, and significant increases in urinary albumin excretion occurred during the peptide infusion. Thus, the high levels of plasma ANF found in CRF may have a role in the maintenance of sodium balance. In addition, the proteinuric effect may be detrimental to long-term renal function.

Introduction

Sodium balance is maintained in chronic renal failure (CRF) by an increase in fractional excretion of sodium, and it has been suggested that this effect is mediated by an unidentified circulating natriuretic agent.^{1,2} Recently, atrial natriuretic factor (ANF) has been implicated as an important hormone in the control of plasma volume. In healthy subjects, ANF is released by the heart in response to blood volume expansion,³ and low dose intravenous infusion causes a significant natriuresis.^{4,5} Cyclic guanosine monophosphate (cGMP) may act as a 'second messenger' to ANF, and extracellular levels of the nucleotide may act as a guide to the tissue action of ANF.⁶

Plasma immunoreactive ANF values are often raised in patients with CRF who do not require dialysis,^{7,8} and this may be due to a combination of volume overload, decreased renal excretion of the peptide together with the frequent occurrence of cardiac disease in this population. However, the biological effects of these high levels are unknown. In order to study the role of ANF in this disorder we have followed the renal and hormonal effects of intravenous ANF infusion.

Patients and methods

After approval by the Department of Health and the hospitals' ethical committees, 15 patients with stable

CRF were recruited from nephrology clinics, and they gave informed consent. None had valvular heart disease, a history of cardiac ischaemia, nephrotic syndrome or peripheral oedema. They were assigned alternately to have either an ANF or placebo infusion. The active group ($n = 8$, 5 males), had diagnoses of glomerulonephritis (3), chronic interstitial disease (3), hypertension (1) and adult polycystic disease (1). The placebo group ($n = 7$, 3 males) had diagnoses of glomerulonephritis (2), chronic interstitial disease (4) and hypertension (1). One patient in each group took their regular nifedipine on the study day, and one subject in each group stopped a thiazide and beta-blockade two days before the study. Dietary sodium was uncontrolled but the two groups had similar urinary sodium excretion on the day before the experiment (active group, mean $\pm$ s.e.m.: 127 ± 23 mmol and placebo group: 145 ± 23 mmol). The daily protein excretion of the active group was 1.27 ± 0.30 g, and of the placebo group was 1.05 ± 0.33 g.

On the study day they ate a light breakfast which did not include meat. From 0900–1400 h they sat with legs dependent, rising briefly to pass urine every 30 minutes. Between 0900–0930 h they each drank 1 litre of water, and took 200 ml half-hourly thereafter. At 0900 h, sodium aminohippurate (Merck, Sharp and Dohme) and inulin (Laevosan International) were infused via a forearm vein, to achieve plasma levels of 20 mg/l and 250 mg/l respectively and continued for 5 hours. After an equilibration hour, and a further control hour, 50 μ g of ANF 99-126

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(Bissendorf Peptide) dissolved in 100 ml 0.9% saline was administered between 1100 and 1300 h into the same vein (an average rate of 1.8 pmol/kg/min). For the placebo infusions, only saline was given. Blood was drawn from the opposite arm at the midpoint of each urine collection. The final hour between 1300 and 1400 h served as a recovery hour.

ANF was measured by radioimmunoassay (RIA) using a polyclonal antibody (Peninsula Laboratories) directed to the biologically active carboxy-terminal of 99-126 ANF. The peptide was extracted from the plasma using octadecylsilica, and the recovery of ANF added to plasma was $70 \pm 5\%$ (mean $\pm$ s.d.). The minimum detectable plasma level was 1 pmol/l, and the coefficient of variation of the RIA was 10%.⁹ Healthy plasma contains two components of the ANF 126 amino-acid prohormone, namely the 1-98 and 99-126 molecules.¹⁰ Chromatography of uraemic plasma shows that 99-126 ANF is the major circulating immunoreactive C-terminal component of the propeptide, although a low concentration of an antiparallel dimer of 99-126 can also be identified.¹⁰ Antisera directed to the carboxy-terminal of 99-126 ANF may cross-react with this larger molecule¹¹ so that the basal immunoreactive ANF measured in this study may be a slight overestimate of true 99-126 ANF values. Although we have not performed chromatograms of plasma extracts from our patients, serial dilutions parallel the 99-126 ANF standard curve, suggesting that the measured immunoreactive ANF corresponded to the 99-126 molecule. Healthy, sitting plasma 99-126 ANF ($n = 24$) in our laboratory was (mean $\pm$ s.e.m.) 6.9 ± 0.8 pmol/l.

Plasma renin activity (PRA) was measured by RIA of angiotensin I generated by plasma incubation at 37°C.⁹ The healthy mean $\pm$ s.e.m. of 18 adults was 1.21 ± 0.16 nmol/h/l. Plasma aldosterone was measured by a commercial RIA (CIS) after plasma extraction.¹² The healthy mean $\pm$ s.e.m. of 18 adults was 258 ± 67 pmol/l. Cyclic GMP was measured by RIA,⁵ and the healthy plasma mean $\pm$ s.e.m. of 18 adults was 1.6 ± 0.2 nmol/l. Urinary albumin was measured with a commercial RIA (Pharmacia).

Sodium and potassium were measured by flame photometer and plasma albumin by the bromocresol green method. Inulin and PAH clearances were taken to equal the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) respectively. No correction was made for tubular extraction efficiency of PAH which was unknown in these patients. One observer measured the blood pressure with a mercury sphygmomanometer every 30 minutes.

Unless otherwise stated results are given as mean $\pm$ s.e.m. Because of the wide variation between subjects in basal GFR, sodium and albumin excretion, these responses were expressed as percentages of the values found during the half-hour basal period

immediately before the start of the ANF or placebo infusion. Student's unpaired *t*-tests were applied between mean active and placebo values at each time point, and upon the difference in incremental areas following the second basal sample. Significance was determined at the 1:20 level.

Results

Basal values in the two groups were similar and are shown in Table I. Significant differences at identical time points are shown in Figure 1, and significant differences in incremental areas are identified in the text.

During the active infusion the mean plasma ANF rose four-fold, and rapidly returned to basal levels in the recovery hour. Basal plasma cGMP was elevated compared to healthy subjects, and in the active group the mean level increased two-fold. Despite a rise of absolute urinary cGMP excretion from 140 ± 30 to 396 ± 87 pmol/min, the fractional excretion of the nucleotide did not change significantly (from 115 ± 19 to $104 \pm 9\%$). The incremental areas for plasma ANF, cGMP and urinary cGMP were significantly greater in the active versus the placebo groups (all $P < 0.01$).

Plasma aldosterone tended to fall during the ANF infusion, and then rose in the recovery hour. Plasma aldosterone was constant in the placebo study but the difference between groups was not significant. PRA showed a tendency to fall throughout the study in both groups.

In the active group the mean GFR tended to rise but the individual absolute increments were highly variable, ranging from 2–20 ml/min. However, the difference of incremental areas of the two groups failed to reach significance ($P = 0.07$). ERPF was constant in both groups.

In the active group urinary sodium increased slowly to reach a peak of $168 \pm 20\%$ of basal values in the final half-hour of the ANF infusion, and by the end of the recovery hour had returned to basal values. In this small group, there was no relation between the absolute rise in sodium excretion rate and the initial GFR. Compared to placebos, there was a highly significant increase in incremental area ($P < 0.001$). During the ANF infusion, fractional excretion of sodium tended to rise and then it fell rapidly in the recovery hour to 50% of basal values. In contrast, in the placebo group, there was a steady fall throughout the experiment of both absolute and fractional sodium excretion. Urine potassium excretion remained constant in both groups.

In the active group urinary albumin excretion rate increased in all patients to a peak mean of $280 \pm 110\%$ of basal values during the second hour of the ANF infusion, and then returned to basal values in the

Table I Basal values

	Active group (n = 8)	Placebo group (n = 7)
Age (years)	40 ± 4	41 ± 5
Mean BP (mmHg)	96 ± 4	97 ± 7
Pulse (bpm)	64 ± 6	62 ± 2
<i>Plasma indices</i>		
Sodium (mmol/l)	136 ± 1	134 ± 1
Potassium (mmol/l)	4.5 ± 0.2	4.5 ± 0.3
Creatinine (μmol/l)	472 ± 100	398 ± 86
Albumin (g/l)	40.1 ± 1.7	40.9 ± 1.0
ANF (pmol/l)	9.6 ± 2.1	10.6 ± 1.8
PRA (nmol/l)	1.24 ± 0.34	1.36 ± 0.59
Aldosterone (pmol/l)	1595 ± 500	1207 ± 425
Cyclic GMP (nmol/l)	7.0 ± 1.4	6.4 ± 1.3
<i>Urine indices</i>		
Cyclic GMP (pmol/min)	140 ± 30	167 ± 44
GFR (ml/min/1.73m ²)	13.5 (8.3–52.4)	23.3 (13.3–45.1)
ERPF (ml/min/1.73m ²)	30 (20–176)	88 (23–157)
Volume (ml/min)	4.4 ± 0.8	4.0 ± 0.7
Sodium (μmol/min)	115 (44–408)	179 (77–224)
Sodium (% GFR)	5.62 ± 1.06	6.13 ± 1.66
Potassium (μmol/min)	51 ± 5	47 ± 8
Albumin (μg/min)	575 (70–1612)	385 (120–1610)
Albumin (% GFR)	0.054 (0.003–0.406)	0.045 (0.006–0.195)

Basal data are given as mean ± s.e.m., unless distribution is non-Gaussian, when values are median (range).

recovery hour. The incremental area was significantly greater than in placebos ($P = 0.02$).

In both groups blood pressure, pulse, plasma electrolytes and plasma albumin were constant. No patient experienced any side effects during either the ANF or placebo infusions.

Discussion

The peak mean plasma value of ANF during the peptide infusion was just above the upper limit of a range of basal levels (1.0–39.0 pmol/l) measured by our laboratory in a larger group of patients with CRF ($n = 36$) of a similar age and degree of renal impairment.⁸ This rise of ANF caused a significant tissue effect as judged by the increases of plasma and urinary cGMP.⁶ At all time periods, in both groups, the ratio of cGMP to inulin clearance was unity and we were unable to detect any nephrogenous nucleotide, confirming previous observations in healthy subjects.¹³ Arterial walls are rich in ANF receptors and may contribute the majority of both plasma and urinary cGMP.⁶ Therefore, urinary cGMP is unlikely to be a marker for the specific renal effects of ANF.

The increase in sodium excretion in the active group

contrasted with the fall which occurred in the placebo group. A similar fall has been recorded in another study, and as a group patients with CRF excrete more sodium at night.¹⁴ The ANF-induced natriuresis suggests that the raised levels of plasma peptide found in some patients with CRF^{7,8} may contribute to sodium balance in this disorder. Although plasma ANF rises in animals with CRF when dietary salt is increased, further studies are required in patients to determine whether the four-fold rise in ANF that we recorded in the active group is compatible with a change caused by dietary sodium loading.

When pharmacological doses of ANF are administered to rats with CRF both sodium excretion and GFR are reported to rise.¹⁶ However, we failed to find a major rise in GFR in our patients. Moreover, low-dose ANF administered to healthy subjects produces natriuresis without a significant rise in filtration rate.^{4,5} Therefore, the importance of a rise in GFR in the ANF-induced natriuresis remains uncertain.

An increase of albuminuria has been recorded after administration of pharmacological doses of ANF to healthy subjects,¹⁷ and during infusion into patients with the nephrotic syndrome.^{5,18} When pharmacological doses of ANF are administered to animals, both the glomerular capillary hydraulic pres-

Figure 1 Mean $\pm$ s.e.m. values of plasma ANF, plasma cyclic GMP, GFR, urinary sodium excretion (U_{Na^+}) and urinary albumin excretion ($U_{albumin}$). Data for GFR and urinary excretions are plotted at the midpoint of each half hour period and are expressed as percentages of the second basal collection. Solid lines represent the active group and short dashes represent the separate placebo group. The asterisks indicate a significant difference between means of each group at each time point. * $P < 0.05$ and ** $P < 0.01$.

sure and permeability of the glomerular barrier increase,¹⁹ and similar mechanisms could have contributed to the rise in albumin excretion in the studies described above.

In patients with chronic glomerulonephritis and pyelonephritis the degree of proteinuria is a marker for the rate of renal deterioration.²⁰ As well as indicating the severity of glomerular structural and functional changes, the enhanced passage of macromolecules across the glomerulus may cause further structural damage.²¹ In animals with partial renal ablation²² and humans with a variety of primary renal diseases²³ the control of arterial hypertension can reduce proteinuria and stabilize renal function, an effect that may be mediated by the lowering of glomerular capillary pressure in surviving nephrons.²²

In patients with CRF there is a positive correlation between plasma ANF and arterial blood pressure,^{7,8} and this may reflect a partial dependence of blood pressure on blood volume.²⁴ It is interesting to speculate that in some patients with CRF, the retention of sodium may lead to both systemic arterial hypertension and a rise of plasma ANF. Both of these changes may then not only serve the homeostatic function of increasing urinary sodium excretion, but could also alter glomerular haemodynamics and increase proteinuria to the detriment of long-term renal function.

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References

1. Slatopolsky, E., Elkan, I.O., Weerts, C. & Bricker, N. Studies on the characteristics of the control system governing sodium excretion in uremic man. *J Clin Invest* 1968, **47**: 521–530.
2. Fine, L.G., Bourgoignie, J.J., Hwang, K.H. & Bricker, N.S. On the influence of the natriuretic factor from patients with chronic uremia on the bioelectric properties and sodium transport of the isolated mammalian collecting tubule. *J Clin Invest* 1976, **58**: 590–597.
3. Sagnella, G.A., Markandu, N.D., Shore, A.C. & MacGregor, G.A. Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. *Lancet* 1985, **ii**: 1208–1211.
4. Richards, A.M., Tonolo, G., Montorsi, P. *et al.* Low dose infusions of 26- and 28-amino acid human atrial natriuretic peptides in normal man. *J Clin Endocrinol Metab* 1988, **66**: 465–473.
5. Woolf, A.S., Lyon, T.L., Hoffbrand, B.I., Cohen, S.L. & Moul, P.J.A. The effects of physiological infusion of atrial natriuretic factor in healthy subjects and patients with the nephrotic syndrome. *Nephron* (in press).
6. Gerzer, R., Weil, J., Strom, T. & Muller, T. Mechanisms of action of atrial natriuretic factor. *Klin Wochenschr* 1986, **64** (Suppl V1): 21–26.
7. Hasegawa, K., Matsushita, Y., Inoue, T., Morii, H., Ishibashi, M. & Yamaji, T. Plasma levels of atrial natriuretic peptide in patients with chronic renal failure. *J Clin Endocrinol Metab* 1986, **63**: 819–822.
8. Woolf, A.S., Kingswood, J.C., Kumar, A., Lyon, T.L., Mansell, M.A. & Moul, P.J.A. Determinants of plasma atrial natriuretic factor in chronic renal failure. *Clin Sci* 1988, **75** (Suppl 19): 48pp.
9. Woolf, A.S. & Moul, P.J.A. Plasma levels of atrial natriuretic peptide in hyperthyroidism. *Clin Endocrinol* 1987, **27**: 721–725.
10. Itoh, H., Nakao, K., Sugawara, A. *et al.* Gamma atrial natriuretic polypeptide (gamma ANP)-derived peptides in human plasma: Cosecretion of N-terminal gamma ANP fragment and alpha ANP. *J Clin Endocrinol Metab* 1988, **67**: 429–437.
11. Itoh, H., Nakao, K., Mukoyama, M. *et al.* Effects of intravenously administered Beta-human atrial natriuretic polypeptide in humans. *Hypertension* 1988, **11**: 697–702.
12. Jones, J.C., Carter, G.D. & MacGregor, G.A. Interference by polar metabolites in a direct radioimmunoassay for plasma aldosterone. *Ann Clin Biochem* 1981, **18**: 54–59.
13. Broadus, A.E., Kaminsky, N.I., Hardman, J.G., Sutherland, E.W. & Liddle, G.W. Kinetic parameters and renal clearances of plasma adenosine 3',5'-monophosphate and guanosine 3',5'-monophosphate in man. *J Clin Invest* 1970, **49**: 2222–2236.
14. Hillier, P., Knapp, M.S. & Cove-Smith, R. Circadian variations in urine excretion in chronic renal failure. *Q J Med* 1980, **XLIX**: 461–478.
15. Smith, S., Anderson, S., Ballerman, B.J. & Brenner, B.M. Role of atrial natriuretic peptide in adaptation of sodium excretion with reduced renal mass. *J Clin Invest* 1986, **77**: 1395–1398.
16. Cole, B.R., Kuhnline, M.A. & Needleman, P. Atriopeptin III. A potent natriuretic, diuretic, and hypotensive agent in rats with chronic renal failure. *J Clin Invest* 1985, **76**: 2413–2415.
17. McMurray, J., Struthers, A.D., Seidelin, P.H. & Howie, J.E.A. The effect of ANF on urinary protein excretion in man. *Clin Sci* 1988, **75** (Suppl 19): 47pp.
18. Zietse, R. & Schalekamp, M.A. Effect of synthetic ANP (102–126) in nephrotic syndrome. *Kidney Int* 1988, **34**: 717–724.
19. Fried, T.A., McCoy, R.N., Osgood, R.W. & Stein, J.H. Effect of atriopeptin II on determinants of glomerular filtration rate in the *in vitro* perfused dog glomerulus. *Am J Physiol* 1986, **250**: F1119–F1122.
20. Williams, P.S., Fass, G. & Bone, J.M. Renal pathology and proteinuria determine progression in untreated mild/moderate chronic renal failure. *Q J Med* 1988, **251**: 343–454.
21. Couser, W.G. & Stilmant, M.M. Mesangial lesions and focal glomerular sclerosis in the aging rat. *Laboratory Investigation* 1975, **33**: 491–501.
22. Meyer, T.W., Anderson, S., Rennke, H.G. & Brenner, B.M. Reversing glomerular hypertension stabilises established glomerular injury in renal ablation. *Hypertens* 1986, **4** (Suppl 5): S239–S241.
23. Alvestrand, A., Gutierrez, A., Bucht, H. & Bergstrom, U. Reduction of blood pressure retards progression of chronic renal failure in man. *Nephrol Dial Transplant* 1988, **3**: 624–631.
24. Tarazi, R.C., Dustan, H.P., Frohlich, E.D., Gifford, R.W. & Hoffman, G.C. Plasma volume and chronic hypertension. *Arch Intern Med* 1970, **125**: 835–842.