Changes in the plasma levels of atrial natriuretic peptides during mineralocorticoid escape in man

FRANCESCO P. CAPPUCCIO, NIRMALA D. MARKANDU, MARTIN G. BUCKLEY, GIUSEPPE A. SAGNELLA, ANGELA C. SHORE AND GRAHAM A. MACGREGOR

Blood Pressure Unit, Department of Medicine, Charing Cross and Westminster Medical School, London

(*Received 7 October 1986; accepted 9 December 1986*)

Summary

1. Plasma levels of atrial natriuretic peptide (ANP) were measured by radioimmunoassay in eight normal healthy volunteers before and during mineralocorticoid escape.

2. Mean plasma ANP on a fixed sodium intake before fludrocortisone was 6.5 ± SEM 1.1 pg/ml. Within 24 h of fludrocortisone administration there was a significant increase in plasma ANP which continued to increase daily reaching a plateau by day 4 (14.9 ± 2.4 pg/ml) to day 7 (15.1 ± 2.6 pg/ml).

3. The rise in plasma ANP was closely related to the amount of sodium retained during the fludrocortisone treatment and the sodium 'escape' occurred by days 4 to 7.

4. These results support the concept that ANP could play an important hormonal role in overcoming the sodium-retaining effects of mineralocorticoids in man.

Key words: atrial natriuretic peptides, mineralocorticoid escape.

Abbreviations: ANG I, angiotensin I; ANP, atrial natriuretic peptide; PRA, plasma renin activity.

Introduction

Administration of aldosterone or other mineralocorticoids is associated with an initial sodium retention of a few days' duration, which is then followed by an 'escape' from the sodium-retaining effects of mineralocorticoids so that sodium excretion is equivalent to sodium intake thereby protecting from continued expansion of extracellular fluid volume and oedema formation [1, 2]. The mechanism underlying this 'escape' has been the subject of great controversy over the last 25 years [2]. The possible contributory role of renal tubular function, haemodynamic and physical factors, the renin–angiotensin system, the renal adrenergic system, kallikrein–kinin and prostaglandins has been investigated and none of these systems is thought to be fully responsible for the sodium 'escape' (for review, see [2]). Furthermore suggestions have been made that an, as yet, unidentified natriuretic hormone is likely to be the mediator of the sodium 'escape' [2–3].

The recent discovery of the atrial natriuretic peptides (ANP) [4–7] which are natriuretic when injected into man [8–11] and the finding that the plasma levels of ANP vary with changes in extracellular volume [12–15] and salt intake [16–18] suggest that they could be an important hormone mediating mineralocorticoid 'escape'. We therefore measured plasma levels of ANP during mineralocorticoid 'escape' in normal man.

This study was presented in part at the Annual Meeting of the British Hypertension Society, Oxford, 23–24 September 1986.

Materials and methods

Subjects

Eight young (19–22 years) white, healthy, male normotensive subjects were studied on a diet provided by the metabolic ward kitchen, which contained 150 mmol of sodium/day and 80 mmol of potassium/day supplemented by 12 Slow-Sodium tablets/day (CIBA: 10 mmol of NaCl/tablet) to give a

Correspondence: Dr G. A. MacGregor, Blood Pressure Unit, Department of Medicine, Charing Cross and Westminster Medical School, London W6 8RF.

total sodium intake of 270 mmol/day. Fluid intake was *ad libitum*. After a 1 week control period, subjects were given fludrocortisone acetate (Florinef, Squibb), 8 $\mu g\ day^{-1}\ kg^{-1}$ body weight orally, in a single morning dose for 10 days. Informed consent was obtained from each subject and the study was approved by the Charing Cross Hospital Ethical Committee.

Protocol

Throughout the study subjects were allowed to go about their normal activities; they were not admitted to hospital but were discouraged from vigorous exercise. During the study subjects were seen in the Blood Pressure Unit at the same time of the day by the same nurse, in the same room. Blood pressure was measured between 10.00 and 12.00 hours in the same arm with a semi-automatic ultrasound sphygmomanometer (Arteriosonde) [19] with attached recorder. The measurements were therefore free from observer bias. Supine and standing blood pressures were taken as the mean of five readings obtained at 1–2 min intervals with the subject in the corresponding position. Supine blood pressure was measured before standing blood pressure. Pulse rate was measured with a Cambridge 3048 pulse monitor. Body weight was recorded at each visit, in the morning, the subjects wearing indoor clothing and without shoes. Continuous 24 h urine collections were obtained throughout the study for measurements of urinary volume, sodium, potassium and creatinine. Urinary electrolytes were measured by flame photometry. Venous blood was taken without stasis and in fasting conditions after the subject had been sitting upright for 10 min between 10.00 and 12.00 hours on days 4 and 7 of the control period, on days 1, 2, 3, 4, 7 and 9 of fludrocortisone administration and on days 1 and 4 after withdrawal for measurement of plasma electrolytes, urea, creatinine, calcium and phosphate, glucose, packed cell volume, plasma renin activity (PRA) and aldosterone. PRA and plasma aldosterone were measured by radioimmunoassay [20, 21].

ANP assay

Plasma ANP was measured by radioimmunoassay as previously described [16]. Venous blood was collected in polypropylene tubes containing ethylenediaminetetra-acetate (potassium salt) and aprotinin (Bayer: 2000 k.i.u./10 ml of blood) and immediately centrifuged at 4°C. The plasma was removed and stored at −20°C until assayed. Immunoreactive ANP was extracted from plasma by passage through C-18 octadecyl silica cartridges (Sep-Pak C-18, Waters, Milford, MA, U.S.A.) previously activated with methanol. Rabbit anti-ANP was obtained from Peninsula Laboratories Europe Ltd (Merseyside, U.K.) and rat ^{125}I-ANP (2000 Ci/mmol) was purchased from Amersham International (Amersham, U.K.). Standard curves were constructed with synthetic atriopeptin III (Peninsula Laboratories) over a concentration range of 3.9–500 pg/tube. The sensitivity of the assay was 3.9 pg/tube, equivalent to 1.8 pg/ml of plasma. Within-assay and between-assay variation was 10.9 and 19.7% respectively. Plasma samples from the same subject were assayed in the same assay. The recovery of human α-ANP from the Sep-Pak cartridge was $84 \pm 8\%$ ($n = 20$, mean ± SD; [16]). Values reported are not corrected for recovery. Mean plasma immunoreactive ANP in a group of 24 normal subjects on their usual sodium intake was 8.4 pg/ml [22]. Similar, but slightly higher, values (i.e. < 20 pg/ml) in normal subjects have also been reported by other laboratories [15, 17, 23]. However, considerably higher values in normal subjects have also been reported, with levels ranging from above 20 to more than 100 pg/ml [24–28]. There are several explanations for these higher levels. Firstly, there may have been differences in the age and sodium intake of the subjects and in the conditions under which the blood was collected (i.e. supine, standing or sitting), factors which are now known to influence the plasma levels of immunoreactive ANP. Secondly, there are also several important methodological differences amongst the reported radioimmunoassays. These are, in particular: (1) whether measurements were carried out directly or after extraction, usually on Sep-Pak as in the present study: unextracted assays, depending on the source of antibody, may result in much higher levels and this may be due to interfering substances which are removed by the extraction procedure ([23]; G. A. Sagnella *et al.*, unpublished work); (2) whether plasma was extracted without acidification or acidified before extraction since acidification of plasma before extraction markedly increases the measured plasma levels (G. A. Sagnella *et al.*, unpublished work); (3) variable degrees of recovery through the extraction stage and differences in the method of drying down the extracts (i.e. freeze-drying/evaporation at different temperatures), but, since the average recovery reported by the various groups, including ourselves, is around 80%, differences in recovery would not therefore markedly influence the measured levels; (4) the use of different antibodies with different affinities for the various atrial peptides used as standards could also account for some of the variability in the reported levels. An International Collaborative Study for a proposed ANP standard (National Institute of Bio-

logical Standards and Control, Holly Hill, Hampstead, London, U.K.) is now under way. The outcome of this study (which also includes ourselves) should help towards establishing a methodologically uniform radioimmunoassay for ANP in human plasma.

Recently, we have developed a radioreceptor assay for the measurement of plasma ANP using solubilized membranes from bovine adrenal cortex. Plasma levels as measured by this method were in close agreement with the corresponding values as measured by radioimmunoassay (Pearson correlation coefficient = 0.95; $n = 25$ [29]). In view of the previously reported [30] strong correlation between receptor binding on adrenal cortical membranes and biological activity of the atrial peptides, these results [29] indicate that the levels as measured by the radioimmunoassay used in the present study are likely to represent the biologically active peptide.

Analysis of data

All results are given as means ± SEM. Mean arterial pressure was calculated by adding one-third of the pulse pressure to the diastolic pressure. Sodium balance was calculated as the cumulative sum of the differences between the average urinary sodium excretion during the control period and daily urinary sodium excretion whilst on treatment with fludrocortisone. Repeated measures analysis of variance and Student's *t*-test for paired observations were used for the statistical analysis [31] using the North Western Universities' Statistical Package for the Social Sciences [32].

Results

During the baseline control period before the administration of fludrocortisone, urinary sodium excretion was stable with an average value of 242 ± 18 mmol/24 h. During fludrocortisone administration there was a significant fall in urinary sodium excretion as compared with basal levels ($F = 2.6$; $P = 0.022$; Table 1). On day 1 of fludrocortisone administration urinary sodium excretion significantly fell to 154 ± 24 mmol/24 h ($P < 0.01$) and remained significantly reduced on day 2 (187 ± 22 mmol/24 h; $P < 0.05$), thereafter returning to the baseline levels by days 4–7 despite the continued administration of fludrocortisone. Urinary sodium excretion was reduced in all eight subjects during the first 2–3 days of fludrocortisone administration.

Plasma ANP measured on days 4 and 7 of the control period showed similar values with a mean of 6.5 ± 1.1 pg/ml. During fludrocortisone administration there was a progressive rise in ANP during the

TABLE 1. *Urinary volume, sodium, potassium and creatinine before and during fludrocortisone administration in eight normal subjects*

Values are means ± SEM. Statistical significance: *$P < 0.05$, †$P < 0.01$ as compared with the mean value at baseline.

	Baseline						Fludrocortisone acetate									
Days...	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10
Urinary sodium (mmol/24 h)	241 ±24	246 ±31	243 ±15	251 ±27	248 ±28	224 ±22	154† ±24	187* ±22	227 ±14	240 ±31	180* ±28	248 ±20	216 ±18	241 ±26	240 ±14	251 ±20
Urinary potassium (mmol/24 h)	87 ±8	67 ±6	65 ±4	65 ±4	55 ±4	55 ±4	77* ±5	78† ±4	74 ±4	60 ±4	59 ±5	66 ±5	60 ±3	65 ±4	60 ±3	60 ±3
Urinary creatinine (mmol/24 h)	17.4 ±1.3	16.4 ±0.5	17.3 ±1.0	16.3 ±0.5	15.7 ±1.1	15.7 ±1.0	16.9 ±1.6	16.2 ±1.0	16.3 ±0.8	17.5 ±1.0	15.7 ±1.3	16.4 ±1.3	15.4 ±1.6	17.4 ±1.2	17.4 ±1.4	15.9 ±1.4
Urinary volume (litres/24 h)	1.74 ±0.13	1.85 ±0.22	1.50 ±0.14	1.53 ±0.19	1.72 ±0.25	1.71 ±0.20	1.13 ±0.13	1.34 ±0.16	1.44 ±0.16	1.64 ±0.20	1.16 ±0.15	1.48 ±0.22	1.37 ±0.13	1.42 ±0.14	1.50 ±0.18	1.85 ±0.14

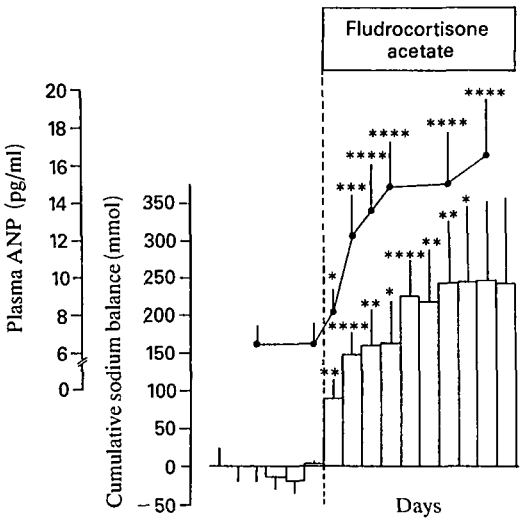

FIG. 1. Plasma levels of ANP and cumulative sodium balance before and during fludrocortisone administration in eight normal subjects. Values are means ± SEM. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.005$, $****P<0.001$ as compared with the mean value at baseline.

first 4 days, reaching a plateau between day 4 and day 7 (Fig. 1). There was a further apparent increase in mean ANP at day 9 (20.0 ± 4.2 pg/ml) which was mainly due to one subject having an extremely high value (43.6 pg/ml) on that day. When this value was excluded the mean value was 16.6 ± 2.9 pg/ml. Plasma ANP increased in all eight subjects during fludrocortisone treatment and in each subject the peak level was at least double the basal level. Daily values from day 1 through to day 9 were all significantly higher than the basal levels ($P<0.05$–0.005; Table 2).

The daily rise in plasma ANP paralleled the return of urinary sodium excretion towards baseline values, so that by days 4–7 urinary sodium excretion matched the sodium excretion during the control period (Table 1). Whilst on fludrocortisone, all subjects were in positive sodium balance and on average retaining more than 240 mmol of sodium (Fig. 1): sodium retention started on day 1 of treatment and continued up to days 4–7 of fludrocortisone administration. The amount of sodium retained (as expressed by the calculated cumulative sodium balance) was closely related to the plasma levels of ANP (Fig. 2*a*).

With the fludrocortisone-induced sodium retention there was a significant ($F=4.3$; $P<0.002$) increase in body weight with an average increase of 1.1 kg by the end of the study (Table 2).

TABLE 2. *Effect of fludrocortisone administration on selected variables in eight normal subjects*

Values are means ± SEM. Statistical significance: $*P<0.05$, $**P<0.01$, $\dagger P<0.005$, $\dagger\dagger P<0.001$ as compared with baseline.

		Fludrocortisone acetate						
	Baseline	Days... 1	2	3	4	7	8	9
Body weight (kg)	66.0 ± 2.1	66.6 ± 2.1**	66.7 ± 2.1*	66.6 ± 2.1	66.4 ± 2.0	66.9 ± 2.1*	67.2 ± 2.1*	67.1 ± 2.1*
Plasma ANP (pg/ml)	6.5 ± 1.1	8.2 ± 1.2*	12.2 ± 2.3**	13.6 ± 2.6†	14.9 ± 2.4†	15.1 ± 2.6†	—	16.6 ± 2.9†‡
PRA (ng of ANG I h^{-1} ml^{-1})	2.60 ± 0.38	1.47 ± 0.32†	1.04 ± 0.26†	0.56 ± 0.14†	0.87 ± 0.28††	0.36 ± 0.14†	—	0.31 ± 0.09†
Aldosterone (pmol/l)	376 ± 46	203 ± 31*	178 ± 27**	131 ± 10†	129 ± 10†	105 ± 8††	—	108 ± 9††
Plasma sodium (mmol/l)	139.9 ± 0.52	140.0 ± 0.60	141.2 ± 0.45	142.2 ± 0.75	141.7 ± 0.99	143.0 ± 1.44	—	140.6 ± 1.07
Plasma potassium (mmol/l)	3.83 ± 0.18	3.75 ± 0.08	3.57 ± 0.10	3.52 ± 0.08	3.57 ± 0.07	3.48 ± 0.06*	—	3.36 ± 0.07*
Systolic blood pressure (mmHg)	116.2 ± 2.4	120.4 ± 4.6	121.4 ± 3.8	122.9 ± 2.8	119.7 ± 3.1	120.2 ± 2.7	—	125.0 ± 4.9
Diastolic blood pressure (mmHg)	66.0 ± 2.2	66.0 ± 1.9	70.7 ± 2.2	67.1 ± 3.8	65.6 ± 3.5	71.2 ± 3.6	—	67.9 ± 4.2
Mean blood pressure (mmHg)	82.7 ± 1.8	84.1 ± 1.7	87.6 ± 1.7	85.7 ± 2.7	83.7 ± 2.2	87.6 ± 2.5	—	86.9 ± 3.4

‡$n = 7$.

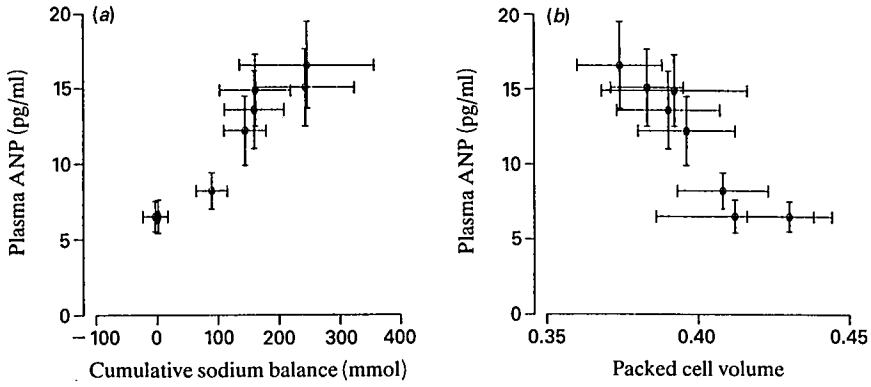

FIG. 2. Daily mean plasma levels of ANP vs daily mean cumulative sodium balance (*a*) and mean packed cell volume (*b*) in eight normal subjects during fludrocortisone administration. Values are means ± SEM.

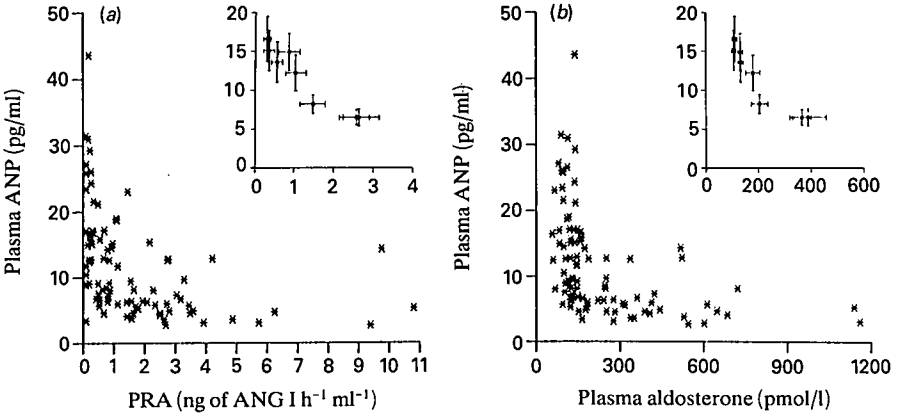

FIG. 3. Plasma levels of ANP vs PRA (*a*) and plasma aldosterone (*b*) in eight normal subjects during fludrocortisone administration. The insets are plots of daily means ± SEM.

During fludrocortisone administration, there was a significant decrease in packed cell volume from a control value of 0.43 ± 0.01 to 0.39 ± 0.02 on day 2 ($P < 0.01$) reaching 0.38 ± 0.01 ($P < 0.001$) by day 7. When the mean values of packed cell volume were plotted against the mean values of ANP there was a trend for an inverse relationship (Fig. 2*b*).

PRA and plasma aldosterone levels are shown in Table 2. Mean PRA during the control period was 2.60 ± 0.38 ng of angiotensin I (ANG I) h^{-1} ml^{-1}. During fludrocortisone administration there was a progressive fall in PRA in all subjects which reached maximum suppression between day 7 and day 9 ($F = 15.1$; $P < 0.001$). The mean level of plasma aldosterone during the control period was 376 ± 46 pmol/l. During fludrocortisone administration there was a progressive fall in plasma aldosterone in all subjects which reached maximum suppression between day 7 and day 9 ($F = 16.5$; $P < 0.001$).

A detailed correlation analysis between plasma ANP and either PRA or plasma aldosterone throughout the study period was not carried out because of intercorrelations between each of these variables on the different days. Nevertheless, when all individual values of ANP were plotted against either PRA (Fig. 3*a*) or aldosterone (Fig. 3*b*) there was an inverse relationship; in other words high values of ANP were associated with low values of PRA and plasma aldosterone.

During the control period urinary potassium excretion was stable ($F = 1.9$; $P =$ NS) with an average value of 66 ± 3 mmol/24 h. After fludrocortisone there was a significant ($F = 3.5$; $P = 0.002$)

but transient increase in potassium excretion on day 1 and day 2 as compared with control values; thereafter the potassium excretion was not statistically different from the control period, despite the continued administration of fludrocortisone (Table 1).

Throughout the whole study there were no significant changes in urinary volume and creatinine (Table 1).

During fludrocortisone treatment plasma sodium increased gradually but did not reach statistical significance ($F = 1.8$; P = NS), whereas plasma potassium decreased significantly ($F = 3.8$; $P = 0.004$; Table 2). During the study there were no significant changes in plasma creatinine and glucose (data not shown).

Supine and standing blood pressures did not change significantly during the study (supine systolic, $F = 1.16$; diastolic, $F = 0.95$); there was also no significant change in mean blood pressure ($F = 1.27$; Table 2). However, a tendency for a rise in blood pressure was observed on day 2 and day 7. No significant changes in heart rate were seen throughout the study (data not shown).

Body weight, plasma ANP, PRA and plasma aldosterone were also measured after stopping fludrocortisone in seven of the eight subjects studied. All subjects showed weight loss, a fall in the plasma levels of ANP and a rise in PRA and plasma aldosterone (Fig. 4), all these variables returning towards the baseline values by day 4 of withdrawal (Table 3).

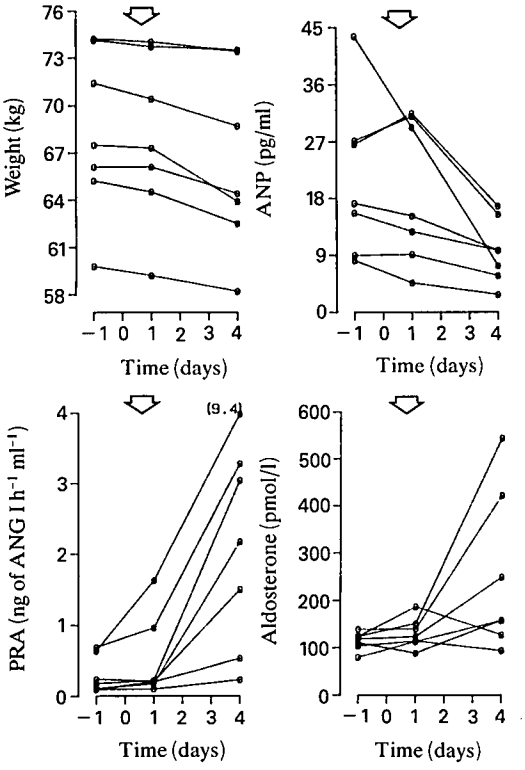

FIG. 4. Body weight, plasma ANP, PRA and plasma aldosterone after withdrawal (arrow) of fludrocortisone in seven normal subjects.

Discussion

Our results clearly demonstrate that mineralocorticoid administration to normal man causes an increase in the plasma level of ANP. The increase in plasma ANP closely paralleled the amount of sodium retained and reached a plateau at the time of mineralocorticoid escape, i.e. when urinary sodium excretion returned to control values. Changes in plasma ANP were also positively associated with changes in weight but inversely with packed cell volume, PRA and plasma aldosterone. The association between these changes in plasma ANP and the above phenomena clearly suggest that the atrial peptides could be playing an important hormonal role in regulating sodium excretion during mineralocorticoid administration in man.

The mechanism whereby the sodium-retaining effect of mineralocorticoids is overcome is not clear, in spite of many studies over the last 25 years

TABLE 3. *Effect of fludrocortisone withdrawal on selected variables in seven normal subjects*

Values are means ± SEM. Statistical significance: *$P < 0.05$, †$P < 0.02$, ‡$P < 0.005$ as compared with day 9.

	Fludrocortisone	Off fludrocortisone	
	Day 9	Day 1	Day 4
Body weight (kg)	68.3 ± 2.0	67.9 ± 2.0†	66.4 ± 2.1‡
Plasma ANP (pg/ml)	21.1 ± 4.7	19.0 ± 4.3	9.6 ± 1.9*
PRA (ng of ANG I h^{-1} ml^{-1})	0.32 ± 0.09	0.50 ± 0.22	3.11 ± 1.35
Plasma aldosterone (pmol/l)	114 ± 7	131 ± 12	251 ± 64

(for review, see [2]). Increased glomerular filtration rate has been suggested to be responsible for the escape during chronic deoxycorticosterone acetate administration [33]. However, the escape can occur in the absence of changes in glomerular filtration rate [34]. The sodium escape is also independent of changes in arterial pressure [35], and changes in the renin–angiotensin system are unlikely to be the major contributor to the sodium escape [36]. The renal adrenergic system is also unlikely to be playing a major role as sodium escape can also occur in the absence of both afferent and efferent renal neural pathways [34, 35]. A slight but not significant fall in urine free dopamine has been described during mineralocorticoid escape in man [37] and changes in prostaglandin metabolism are also associated with the mineralocorticoid escape [38]; however, the escape cannot be blocked by pretreatment with inhibitors of prostaglandin synthetase [39]. Several workers therefore concluded that an unidentified natriuretic hormone might be responsible for the escape [3, 40, 41].

The recent discovery of ANP with potent diuretic and natriuretic properties [4–7] suggests that they could play an important role in the regulation of sodium and water balance and may be important in the sodium escape phenomenon. The presence of specific receptors for atrial peptides in renal tissue [42–44], the findings that ANP are natriuretic when infused into normal man [8–11] and that plasma levels of ANP change with changes in extracellular volume [12–14] such as occurs with saline infusion [15, 16] or alterations in dietary sodium intake [16–18, 45], strongly suggest that the atrial peptides may be directly involved in the control of sodium and water balance.

The increases in the plasma levels of ANP achieved in the present study are of the same order of magnitude of those achieved with other manoeuvres, such as saline infusion [15, 16], high sodium intake [16–18, 45] and head-out water immersion [46]. Recently, Anderson *et al.* [47] have demonstrated that plasma levels of ANP achieved with saline infusion are similar to those measured during a low dose infusion of ANP which causes natriuresis in man, supporting the concept that ANP is natriuretic at levels circulating in man. In rats, Hirth *et al.* [48] have also shown that the volume expansion-induced diuresis and natriuresis, which are associated with increase in circulating atrial peptides, are both blocked by specific monoclonal antibodies directed against atriopeptin III. In the present study plasma levels of ANP were doubled during fludrocortisone administration and it is likely that they were, at least in part, responsible in overcoming the sodium-retaining effect of the fludrocortisone.

Studies of mineralocorticoid escape have shown volume expansion in both animals and man [49, 50] and a secondary increase in central venous pressure due to sodium and water retention [51]. Central blood volume expansion and increases in intra-atrial pressure can be the stimulus for the release of ANP [12, 14, 15, 24, 46, 52]. In the present study, the increase in body weight and cumulative sodium balance and the decrease in packed cell volume during fludrocortisone administration are consistent with an increase in blood volume which is likely to cause a rise in intra-atrial pressure and thereby increase the release of atrial peptides.

The sodium escape phenomenon during mineralocorticoid administration in animals is not dependent on the associated suppression of the renin–angiotensin system [36]. Nevertheless the fall in PRA and aldosterone that occurs with the sodium retention could play a contributory role. Atrial peptides under certain circumstances can inhibit the release of renin and aldosterone [47, 53], and this could indirectly also contribute to the natriuretic actions of the atrial peptides.

Our results, although not a direct proof, strongly suggest that the ANP mediate, at least in part, mineralocorticoid escape in man. More importantly they suggest that the atrial peptides could play a critical role in protecting from progressive volume expansion and oedema in pathophysiological conditions where there is excess circulating mineralocorticoids such as primary aldosteronism and heart failure, conditions where raised plasma levels of ANP have already been reported [24, 52, 54].

Acknowledgments

F.P.C. is the recipient of a grant from the Ministero Pubblica Istruzione Repubblica Italiana. G.A.M. is a Wellcome Trust Senior Lecturer. This study was supported by the National Kidney Research Fund (U.K.).

References

1. August, J.L., Nelson, D.H. & Thorn, G.W. (1958) Response of normal subjects to large amounts of aldosterone. *Journal of Clinical Investigation*, **37**, 1549–1555.
2. Knox, F.G., Burnett, J.C., Jr, Kohan, D.E., Spielman, W.S. & Strand, J.C. (1980) Escape from the sodium-retaining effects of mineralocorticoids. *Kidney International*, **17**, 263–276.
3. Poston, L., Wilkinson, S., Sewell, R.B. & Williams, R. (1982) Sodium transport during the natriuresis of volume expansion; a study using peripheral blood leucocytes. *Clinical Science*, **63**, 243–249.
4. Sagnella, G.A. & MacGregor, G.A. (1984) Cardiac peptides and the control of sodium extraction. *Nature (London)*, **309**, 666–667.

5. de Bold, A.J. (1985) Atrial natriuretic factor: a hormone produced by the heart. *Science*, **30**, 767-770.
6. Needleman, P., Adams, S.P., Cole, B.R., Currie, M.G., Geller, D.M., Michener, M.L., Saper, C.B., Schwartz, D. & Standaert, D. (1985) Atriopeptins as cardiac hormones. *Hypertension*, **7**, 469-482.
7. Laragh, J.H. (1985) Atrial natriuretic hormone, the renin-aldosterone axis, and blood pressure electrolyte homeostasis. *New England Journal of Medicine*, **313**, 1330-1340.
8. Richards, A.M., Nicholls, M.G., Ikram, H., Webster, M.W.I., Yandle, T.G. & Espiner, E.A. (1985) Renal haemodynamic and hormonal effects of human alpha atrial natriuretic peptide in healthy volunteers. *Lancet*, **i**, 545-549.
9. Anderson, J.V., Struthers, A.D., Christofides, N.D. & Bloom, S.R. (1986) Atrial natriuretic peptides: an endogenous factor enhancing sodium excretion in man. *Clinical Science*, **70**, 327-331.
10. Kuribayashi, T., Nakazato, M., Tanaka, M., Nagamine, M., Kurihara, T., Kangawa, K. & Matsuo, H. (1985) Renal effects of human α-atrial natriuretic polypeptide. *New England Journal of Medicine*, **312**, 1456.
11. Biollaz, J., Nussberger, J., Porchet, M., Brunner-Ferber, F., Otterbein, E.S., Gomez, H., Waeber, B. & Brunner, H.R. (1986) Four-hour infusions of synthetic atrial natriuretic peptide in normal volunteers. *Hypertension*, **8** (Suppl. II), 96-105.
12. Lang, R.E., Tholken, H., Ganten, D., Luft, F.C., Ruskoaho, H. & Unger, T. (1985) Atrial natriuretic factor — a circulating hormone stimulated by volume loading. *Nature (London)*, **314**, 264-266.
13. Pettersson, A., Hedner, J., Ricksten, S.E., Towle, A.C. & Hedner, T. (1986) Acute volume expansion as a physiological stimulus for the release of atrial natriuretic peptides in the rat. *Life Sciences*, **38**, 1127-1133.
14. Katsube, N., Schwartz, D. & Needleman, P. (1985) Release of atriopeptin in the rat by vasoconstrictors or water immersion correlates with changes in right atrial pressure. *Biochemical and Biophysical Research Communications*, **133**, 937-944.
15. Anderson, J.V., Donckier, J., McKena, V.J. & Bloom, S.R. (1986) The plasma release of atrial natriuretic peptide in man. *Clinical Science*, **71**, 151-156.
16. Sagnella, G.A., Markandu, N.D., Shore, A.C. & MacGregor, G.A. (1985) Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. *Lancet*, **ii**, 1208-1211.
17. Hollister, A.S., Tanaka, I., Imada, T., Onrot, J., Biaggioni, I., Robertson, D. & Inagami, T. (1986) Sodium loading and posture modulate human atrial natriuretic factor plasma levels. *Hypertension*, **8** (Suppl. II), 106-111.
18. Shenker, Y., Sider, R.S., Ostafin, E.A. & Grekin, R.J. (1985) Plasma levels of immunoreactive natriuretic factor in healthy subjects and in patients with oedema. *Journal of Clinical Investigation*, **76**, 1684-1687.
19. George, C.F., Lewis, P.J. & Petrie, A. (1975) Clinical experience with use of the ultrasound sphygmomanometer. *British Heart Journal*, **37**, 804-807.
20. Roulston, J.E. & MacGregor, G.A. (1978) Measurement of plasma renin activity by radioimmunoassay after prolonged cold storage. *Clinica Chimica Acta*, **88**, 45-48.
21. James, V.H.T. & Wilson, G.A. (1976) Determination of aldosterone in biological fluids. In: *Assay of Drugs and Other Trace Compounds in Biological Fluids. Methodological Development in Biochemistry*, vol. 5, pp. 149-158. Ed. Reid, E. Elsevier, Amsterdam.
22. Sagnella, G.A., Markandu, N.D., Shore, A.C. & MacGregor, G.A. (1986) Raised circulating levels of atrial natriuretic peptides in essential hypertension. *Lancet*, **i**, 179-181.
23. Gutkowska, J., Bonan, R., Roy, D., Bourassa, M., Garcia, R., Thibault, G., Genest, J. & Cantin, M. (1986) Atrial natriuretic factor in human plasma. *Biochemical and Biophysical Research Communications*, **139**, 287-295.
24. Richards, A.M., Cleland, J.G.F., Tonolo, G., McIntyre, G.D., Leckie, B.J., Dargie, H.J., Ball, S.G. & Robertson, J.I.S. (1986) Plasma α natriuretic peptide in cardiac impairment. *British Medical Journal*, **293**, 409-412.
25. Wilkins, M.R., Wood, J.A., Adu, D., Lote, C.J., Kendal, M.J. & Michael, J. (1986) Change in plasma immunoreactive atrial natriuretic peptide during sequential ultrafiltration and hemodialysis. *Clinical Science*, **71**, 157-160.
26. Yandle, T.G., Espiner, E.A., Nicholls, M.G. & Duff, H. (1986) Radioimmunoassay and characterization of atrial natriuretic peptide in human plasma. *Journal of Clinical Endocrinology and Metabolism*, **63**, 72-727.
27. Jappner, H., Brabant, G., Kapteina, U., Kirschner, T., Klein, H. & Hesch, R.D. (1986) Direct radioimmunoassay for human atrial natriuretic peptide (hANP) and its clinical evaluation. *Biochemical and Biophysical Research Communications*, **139**, 1215-1223.
28. Ogihara, T., Shima, J., Hara, H., Kumahara, Y., Kangawa, K. & Matsuo, H. (1986) Changes in human plasma atrial natriuretic polypeptide concentration in normal subjects during passive leg raising and whole body tilting. *Clinical Science*, **71**, 147-150.
29. Sagnella, G.A., Buckley, M.G., Markandu, N.D. & MacGregor, G.A. (1987) Atrial natriuretic peptide (ANP) in human plasma: comparison of radioreceptor vs radioimmunoassay. *Clinical Science*, **72** (Suppl. 16), 62P.
30. De Lean, A., Thibault, G., Seidan, N.G., Lazure, C., Gutkowska, J., Chretien, M., Genest, J. & Cantin, M. (1985) Structure-activity relationships of atrial natriuretic factor (ANF) III: correlation of receptor affinity with relative potency on aldosterone production in zona glomerulosa cells. *Biochemical and Biophysical Research Communications*, **132**, 360-367.
31. Snedecor, G.W. & Cochran, W.G. (1980) *Statistical Methods*. Iowa State University Press, Ames, Iowa.
32. SPSS Inc. (1983) *SPSS X User's Guide*. McGraw-Hill Book Company, New York.
33. Schnermann, J., Hermle, M., Schmidmeier, E. & Dahlheim, H. (1975) Impaired potency for feedback regulation of glomerular filtration rate in DOCA escaped rats. *Pflügers Archiv*, **358**, 325-338.
34. Davis, J.O., Holman, J.E., Carpenter, C.C.J., Urquhart, J. & Higgins, J.T., Jr (1964) An extra-adrenal factor essential for chronic renal sodium retention in the presence of increased sodium-retaining hormone. *Circulation Research*, **14**, 17-31.
35. Higgins, J.T., Jr (1970) Escape from sodium-retaining effects of deoxycorticosterone in hypotensive and hypertensive dogs. *Proceedings of the Society for Experimental Biology and Medicine*, **134**, 768-772.
36. Johnston, C.I., Davis, J.O., Robb, C.A. & Mackensie, J.W. (1968) Plasma renin in chronic experimental

heart failure and during renal sodium escape from mineralocorticoids. *Circulation Research*, **22**, 113–125.
37. Oates, N.S., Perkins, C.M. & Lee, M.R. (1980) The effect of mineralocorticoid administration on urine free dopamine in man. *Clinical Science*, **58**, 77–82.
38. Youngberg, S.P., Marchard, G.R., Romero, J.C. & Knox, F.G. (1977) Mineralocorticoid escape: the role of prostaglandins. *Federation Proceedings*, **36**, 627.
39. Zipser, R.D., Zia, P., Stone, R.A. & Horton, R. (1978) The prostaglandin and kallikrein-kinin systems in mineralocorticoid escape. *Journal of Clinical Endocrinology and Metabolism*, **47**, 996–1001.
40. Buchalew, V.M., Jr & Lancaster, C.D., Jr (1972) The association of a humoral sodium transport inhibitory activity with renal escape from chronic mineralocorticoid administration in the dog. *Clinical Science*, **42**, 69–78.
41. de Wardener, H.E. (1977) Natriuretic hormone. *Clinical Science and Molecular Medicine*, **53**, 1–8.
42. Napier, M.A., Vandlen, R.L., Albers Schonberg, G., Nutt, R.F., Brady, S., Lyle, T., Winquist, R., Faison, E.P., Heinel, L.A. & Blaine, E.H. (1984) Specific membrane receptors for atrial natriuretic factor in renal and vascular tissues. *Proceedings of the National Academy of Sciences U.S.A.*, **81**, 5946–5950.
43. Ballerman, B.J., Hooever, R.L., Karnovsky, M.J. & Brenner, B.M. (1985) Physiologic regulation of atrial natriuretic peptide receptors in rat renal glomeruli. *Journal of Clinical Investigation*, **76**, 2049–2056.
44. Carrier, F., Thibault, G., Schiffrin, E.L., Garcia, R., Gutkowska, J., Cantin, M. & Genest, J. (1985) Partial characterization and solubilization of receptors for atrial natriuretic factor in rat glomeruli. *Biochemical and Biophysical Research Communications*, **132**, 666–673.
45. Sagnella, G.A., Markandu, N.D., Shore, A.C., Forsling, M.L. & MacGregor, G.A. (1987) Plasma atrial natriuretic peptide: its relationship to changes in sodium intake, plasma renin activity and aldosterone in man. *Clinical Science*, **72**, 25–30.
46. Anderson, J.V., Millar, N.D., O'Hara, J.P., Mackenzie, J.C., Corral, R.J.M. & Bloom, S.R. (1986) Atrial natriuretic peptide: physiological release associated with natriuresis during water immersion in man. *Clinical Science*, **71**, 319–322.
47. Anderson, J.V., Donckier, J., Payne, N.N., Beacham, J., Slater, J.D.H. & Bloom, S.R. (1987) Atrial natriuretic peptide: evidence of action as a natriuretic hormone at physiological plasma concentrations in man. *Clinical Science*, **72**, 305–312.
48. Hirth, C., Stasch, J.P., John, A., Kazda, S., Morich, F., Neuser, D. & Wohlfeil, S. (1986) The renal response to acute hypervolemia is caused by atrial natriuretic peptides. *Journal of Cardiovascular Pharmacology*, **8**, 268–275.
49. Mitchell, J., Ling, W.D. & Bohr, D.F. (1984) Deoxycorticosterone acetate hypertension in the sheep. *Journal of Hypertension*, **2**, 473–478.
50. Whitworth, J.A., Butkus, A., Coghlan, J.P., Denton, D.A., Mills, E.H., Spence, C.D. & Scoggins, B.A. (1986) 9-Alpha fluorocortisol-induced hypertension: a review. *Journal of Hypertension*, **4**, 133–139.
51. Distler, A. & Philipp, T. (1979) Haemodynamic studies on the blood pressure-raising effect of mineralocorticoids. *Klinische Wochenschrift*, **7**, 1177–1183.
52. Raine, A.E.G., Erne, P., Burgisser, E., Muller, F.B., Bolli, P., Burkart, F. & Buhler, F.R. (1986) Atrial natriuretic peptide and atrial pressure in patients with congestive heart failure. *New England Journal of Medicine*, **315**, 533–537.
53. Anderson, J.V., Struthers, A.D., Payne, N.N., Slater, J.D.H. & Bloom, S.R. (1986) Atrial natriuretic peptide inhibits the aldosterone response to angiotensin II in man. *Clinical Science*, **70**, 507–512.
54. Tunny, T.J., Higgins, B.A. & Gordon, R.D. (1986) Plasma levels of atrial natriuretic peptide in man in primary aldosteronism, in Gordon's syndrome and in Bartter's syndrome. *Clinical and Experimental Pharmacology and Physiology*, **13**, 341–345.