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

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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±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
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 μg 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 samples were 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 125I-ANP (2000 Ci/mmoll 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 ± 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-
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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
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 2a).

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).
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. 2b).

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. 3a) or aldosterone (Fig. 3b) 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 \((F = 1.8; P = \text{NS})\), whereas plasma potassium decreased significantly \((F = 3.8; P = 0.004; \text{Table 2})\). 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 \((F = 1.27; \text{Table 2})\). 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).

**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.

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<td>Body weight (kg)</td>
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<td>66.4 ± 2.1‡</td>
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<td>Plasma ANP (pg/ml)</td>
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<td>9.6 ± 1.9*</td>
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<td>PRA (ng of ANG 1 h⁻¹ ml⁻¹)</td>
<td>0.32 ± 0.09</td>
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<td>3.11 ± 1.35</td>
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<td>Plasma aldosterone (pmol/l)</td>
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Plasma ANP and mineralocorticoid escape

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 intratrial 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].

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References


