Plasma Levels of Atrial Natriuretic Peptide Are Raised in Essential Hypertension During Low and High Sodium Intake*

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Summary. Plasma levels of α-human atrial natriuretic peptide (hANP) were measured in 17 patients with primary hypertension (11 females, 6 males, aged 22–61; blood pressure systolic 154 ± 7 mmHg, diastolic 92 ± 4 mmHg) and in 9 normotensive controls (4 males, 5 females, aged 20–71; blood pressure systolic 117 ± 4 mmHg, diastolic 76 ± 2 mmHg) during unrestricted sodium diet, at the 4th day of a low sodium intake (40–60 mEq/day) and at the 6th day of sodium loading (280–320 mEq/day) both after an overnight rest and after 4 h of upright posture. In the controls, plasma levels of hANP at 8:00 a.m. were lowered from 73 ± 11 to 49 ± 7 pg/ml during low sodium diet and increased to 128 ± 37 pg/ml after high salt intake. Plasma ANP levels were significantly lower after 4 h of upright posture during unrestricted, low and high sodium intake. In the hypertensive group, plasma ANP levels were elevated during unrestricted diet (203 ± 43 pg/ml), during the low sodium period (139 ± 31 pg/ml), and after high sodium intake (267 ± 63 pg/ml) compared to the controls. All levels were lowered by upright posture. The absolute decrease was more pronounced compared to the normotensives, the relative decline was similar in both groups. In the hypertensives, plasma ANP levels significantly correlate with systolic and diastolic blood pressure ($r = 0.468$, $r = 0.448$, $P < 0.05$) and with urinary aldosterone during unrestricted diet ($r = 0.536$, $P < 0.05$). There was an inverse correlation between plasma ANP levels and plasma renin concentration during low and high sodium intake ($r = -0.469$, $r = -0.496$, $P < 0.05$).

These studies demonstrate raised circulating plasma ANP levels in patients with essential hypertension. The modulation of ANP by different sodium intake and by upright posture is maintained similar to the changes in plasma ANP in normotensive controls. Raised ANP levels in the hypertensives are correlated with low renin secretion and high aldosterone excretion. High ANP levels, therefore, might indicate sodium retention in essential hypertension.

Key words: Human atrial natriuretic peptide – Sodium – Primary hypertension

Abbreviation: ANP = atrial natriuretic peptide

Sodium chloride plays a major role in essential hypertension. Mean daily sodium intake correlates with the incidence of primary hypertension in different populations. Furthermore, reduction in dietary sodium intake or diuretic therapy has been shown to reduce the arterial blood pressure. It has been postulated, that an impaired renal sodium excretion is the primary abnormality in essential hypertension. Urinary sodium output is controlled by glomerular filtration and tubular absorption, the activity of the renin-angiotensin-aldosterone system, and the systemic arterial blood pressure itself. Atrial natriuretic peptide (ANP) is a newly discovered hormonal system involved in the regulation of sodium metabolism. This peptide with 28 amino acids is produced by atrial muscle cells and is released into the circulation after atrial stretch. ANP administered to experimental animal or man rapidly induces natriuresis and lowers arterial blood pressure [4]. Furthermore, ANP has been shown to inhibit renin secretion and aldoster-
one production by the adrenal gland [12]. The secretion of ANP in patients with essential hypertension is therefore of considerable interest. First reports have described increased plasma levels of ANP in patients with essential hypertension [2, 13, 15]. However, this was not confirmed by others [1]. In the present study, we have investigated plasma levels of ANP in essential hypertensives and in normotensives after different dietary sodium intake. The results were correlated with the activity of the renin-aldosterone-system in patients and in controls.

Patients and Methods

A total of 17 patients with essential hypertension (11 females, 6 males, aged 22–61) were included in the study. They were off medication for at least 1 week and they were followed for a period of 2 weeks under clinical conditions. Routine workup included physical examination, electrocardiogram, heart X-ray, blood levels of urea, creatinine, sodium, potassium, glucose, and lipids. Patients with signs of cardiac failure were excluded. The control group consisted of 9 normotensives (4 males, 5 females, aged 20–71; blood pressure systolic 117 +/- 4 mmHg and diastolic 76 +/- 2 mmHg). All patients and control subjects were studied during unrestricted diet, after 4 days on low sodium intake (40–60 mEq/day) and on the 6th day of a high sodium intake (300–320 mEq/day), respectively. At the end of each of the three periods, the 24-h urine was collected for measurement of urinary sodium and potassium output and of urinary aldosterone excretion. Blood samples were drawn after an overnight rest at 8:00 a.m. and after upright posture at 12:00 a.m. for measurements of serum sodium and potassium, plasma ANP levels, plasma-renin-concentration, and serum-aldosterone.

Plasma levels of ANP were measured by radioimmunoassay after extraction of the plasma in SEP-PAK C18 cartridges (Waters Associates, Milford, MA., USA). Columns were equilibrated with acetonitrile (3 ml), water (10 ml), and 10 ml potassium phosphate buffer solution (0.1 M, pH 3.5). Thereafter, plasma samples (2 ml) were transferred to the columns. Plasma proteins were removed by washing with 3 ml buffer and 2 ml water. The ANP-containing fractions were eluted with 3 ml of a mixture of acetonitrile/water (50:50 v/v). The eluate was evaporated for 12 h at 40°C. Dry material was cooled to 4°C and redissolved in 500 µl assay buffer. This assay buffer contained 19 mM monobasic sodium phosphate, 81 mM dibasic sodium phosphate, 50 mM sodium chloride, 0.1% BSA, 0.1% triton-X-100, and 0.01% NaN3. The recovery of labelled ANP tested by the addition of 125I-ANP (Amersham) was in the range of 60%–65%. Recoveries were monitored in each assay and plasma levels of ANP were corrected for the loss of ANP during the extraction.

Extracts of the samples (100 µl) and ANP standards (Bissendorf Peptide, 3002 Wedemark, Germany) were incubated with ANP antiserum (Peninsula Laboratories) and dissolved in 100 µl triton-X-100 (0.1%). After an overnight incubation at 4°C, 125I-ANP (Amersham) was added and the incubation was continued at 4°C for an additional 24-h period.

Separation of bound and free activity was performed on the next day using a double antibody technique with goat antirabbit IgG and normal rabbit serum (Peninsula). Assay buffer was added and samples were centrifuged for 20 min at 1,700 g and 4°C. The supernatant was discharged and the activity of the sediment was recorded in a gamma-counter (Berthold, LB 2101). All samples were done in triplicate. The assay sensitivity allowed measurements of ANP levels above 2 pg per tube. Serum aldosterone levels were measured by radioimmunoassay with a specific antibody produced by IDW, Dreieich. Urine aldosterone-18-glucuronide was measured by RIA after extraction of the urine at pH 1 and paper chromatography [6].

Plasma renin concentration was determined by a method based on an external calibration by the human renin standard of the Medical Research Council [9].

The concentration of sodium and potassium in serum and urine were measured by flame photometry.

Statistical analysis was done using the paired and unpaired t-test and linear regression analysis.

Results

Sodium intake and posture clearly influenced plasma levels of ANP in normotensive controls (Fig. 1). On day 4 of a low sodium diet, plasma ANP was lowered from 73±11 to 49±7 pg/ml at 8:00 a.m. from 48±9 to 38±4 pg/ml at noon compared to values after unrestricted diet (P<0.05). In contrast, plasma ANP levels increased significantly to 128±88 pg/ml and 72±13 pg/ml at 8:00 a.m. and noon, respectively, after 6 days on a high sodium intake (P<0.005). Reciprocal changes in plasma renin concentration and in serum aldosterone were observed under conditions of low and high sodium intake. Serum aldosterone measured at 8:00 a.m. rose from 129±15.9 pg/ml to