Effects of \(\alpha\)-Human Atrial Natriuretic Peptide in Essential Hypertension

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SUMMARY Because there is little published information on the effects of atrial peptides in hypertensive humans, 100 \(\mu\)g of \(\alpha\)-human atrial natriuretic peptide was injected intravenously into six patients with essential hypertension in a double-blind, placebo-controlled study under standardized conditions of body posture and dietary sodium and potassium intake. The peptide increased urine sodium excretion sixfold in the first 30 minutes. Smaller increments occurred in urine volume and in calcium, magnesium, and phosphorus excretion; the rise in urine potassium concentration was not statistically significant. Most of these indices returned to time-matched placebo values within 1 hour, but urine sodium excretion remained high for 2\(\frac{1}{2}\) hours. Arterial pressure fell within 2 minutes of \(\alpha\)-human atrial natriuretic peptide injection, then returned to matching placebo levels by 10 minutes. Conversely, heart rate increased rapidly and remained elevated for 3 hours. The peptide induced a prompt, brief rise in plasma norepinephrine concentration and a more sustained fall in epinephrine and aldosterone levels, but it did not affect plasma renin activity or cortisol concentration. Compared with normotensive volunteers studied previously under the same conditions, the hypertensive subjects had a greater response in urine volume and sodium, calcium, and magnesium excretion but a less sustained fall in arterial pressure. (Hypertension 7: 812-817, 1985)

KEY WORDS • sodium • aldosterone • epinephrine • norepinephrine • arterial pressure • heart rate

Mammalian atria contain natriuretic and vasoactive peptides. There are at least three biologically active peptides in human atria called \(\alpha\)-, \(\beta\)-, and \(\gamma\)-human atrial natriuretic peptides (\(\alpha\)-hANP, \(\beta\)-hANP, and \(\gamma\)-hANP); all three are derived from a common precursor molecule of 151 amino acids. Preliminary experiments suggest that the 28 amino acid \(\alpha\)-hANP may have a more potent natriuretic action than either \(\beta\)-hANP or \(\gamma\)-hANP, but the circulating form (or forms) of atrial peptides has not yet been established. In view of their potent natriuretic and vasodepressor actions, these peptides may play a role in normal body fluid and blood pressure homeostasis. Aberrant response patterns to atrial peptides have been reported in animals with experimental hypertension, but as far as we know, there is no comparable information on their action in human hypertension. The present study analyzed the renal, hemodynamic, and hormone responses to intravenous \(\alpha\)-hANP in six patients with essential hypertension and compared the findings with those previously obtained in normotensive volunteers studied under identical conditions.

Subjects and Methods

We studied six men with essential hypertension, aged 37 to 54 years (mean, 46 yr), weighing 72 to 93 kg (mean, 81 kg). Three were medical or paramedical workers, another had undergone an in-patient research study in the past, but the other two patients were not familiar with the procedures. One had never received antihypertensive treatment, and the other five stopped taking medications (a \(\beta\)-blocker in 4 patients, a thiazide diuretic in 3, \(\alpha\)-methyldopa in 1) 2 to 6 weeks before starting the protocol. All patients had normal plasma levels of urea, creatinine, sodium, and potassium; none had an intercurrent illness; and none had taken medication other than to lower blood pressure.

The protocol, approved by the Ethical Committee of the Canterbury Hospital Board, was identical to that undertaken by six normotensive volunteers approxi-
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In brief, the patients received a-hANP or placebo 1 to 4 weeks apart in a double-blind, randomized fashion on the fourth day of a caffeine-free diet of constant sodium and potassium content (120 mmol/day and 60 mmol/day respectively). Usual daily activities were maintained except on the days of a-hANP or placebo injection, and all subjects slept at home. On the two study days they ate breakfast at 0700 hours, completed a 24-hour urine collection at 0800 hours, and were weighed. A cannula was placed in the brachial artery of the nondominant arm for continuous recordings of pressure and heart rate (using the Oxford technique), and two venous lines were inserted, one for hormone measurements, the other for injection of a-hANP or placebo. From 0830 to 1330 hours the patients were seated in an easy chair apart from brief periods of standing to pass urine every 30 minutes. They drank distilled water equal in volume to the urine passed in the previous 30 minutes plus 50 ml. The air temperature in the study room was between 24 and 27.5 °C. After four 30-minute baseline urine collections, a-hANP (100 μg) or placebo was injected intravenously over 60 seconds. The half hourly urine collections and the arterial pressure recordings were continued, and venous samples were drawn at set intervals for hormone measurement. As each urine sample was passed, volume, pH, and osmolality (Wescor vapor pressure osmometer, Logan, UT, USA) were measured immediately and aliquots were taken for measurements of sodium and potassium (flame photometry), calcium and magnesium (atomic absorption spectrophotometry), phosphorus (Gomori's colorimetric method), and creatinine (Beckman analyzer, Palo Alto, CA, USA).

In each blood sample we measured plasma renin activity, aldosterone, cortisol (enzyme-linked immunosorbant assay), and catecholamines. Samples from the first and second study were run in the same assay. Intraassay variability (coefficient of variability) for individual hormones varied from 5.2% (aldosterone) to 12% (epinephrine).

We prepared a-hANP for injection as in our previous study of normotensive volunteers. Two milligrams of synthetic a-hANP (Bachem, Inc., Torrance, CA, USA) was dissolved in 4 ml of acid saline (0.9% NaCl in 3 mM HCl), made up to a volume of 20 ml with Haemaccel (Behringwerke, AG, Marburg/Lahn, W. Germany), passed through a 0.2-μm Gelman Acrodisc filter (Ann Arbor, MI, USA), and stored in 1-ml aliquots, each containing 100 μg a-hANP, at −70 °C. Placebo consisted of acid saline in Haemaccel, prepared and stored under the same conditions. Recovery of 125I-a-hANP in saline, subjected to the same filtering process, was 97.3 ± 3.3% (SD; n = 10). Sterility of the injectate was confirmed by lack of bacterial growth on incubation using various media at 4 °C, room temperature, and 37 °C for 6 days. We did not perform formal pyrogen testing. Amino acid composition of the a-hANP was confirmed using a Durham amino acid analyzer (Sunnyvale, CA, USA) after hydrolysis with hydrochloric acid under a vacuum.

A paired t test was used for analysis of data within the group of hypertensive patients. Comparisons of results between the hypertensive and normotensive groups were made using the t test or analysis of variance with program P2V from the BMDP package (University of California, Los Angeles, CA, USA). Results are given as means ± SEM.

Results

Details of body weight and arterial pressure measurements together with 24-hour excretion rates of sodium and potassium before the injection of a-hANP and placebo are given in Table 1. The values were similar on the two occasions. The preinjection levels of mean arterial pressure integrated over 1 hour (0930-1030 hours) were 116 ± 4.3 mm Hg before a-hANP and 112.5 ± 2.1 mm Hg before placebo. Both of these values were well above time-matched recordings in the normotensive subjects studied previously (89 ± 2.1 mm Hg and 89 ± 2.5 mm Hg), and there was no overlap in pressures between the two groups. Three hypertensive subjects received a-hANP before placebo, and the sequence was reversed in the other three subjects. The studies were completed without complication, and collection of data was complete. Three patients experienced a brief sensation of facial flushing soon after the injection of a-hANP, and the ears of one were visibly red for about 2 minutes. All reported bladder distention within 30 minutes of a-hANP administration.

Urinary Response

Urine indices in the baseline 30-minute collections (0830-1030 hours) were similar on the two experimental days (Figure 1). In the 30 minutes after a-hANP injection there was a sixfold rise in urine sodium excretion and threefold to fivefold increases in volume and calcium, magnesium, and phosphorus excretion compared with time-matched placebo levels (Figure 1). Most indices had returned to matching placebo values within 1 hour, but sodium excretion remained high for 2½ hours (Figure 1). Urine osmolality fell steadily and similarly on the two experimental days, and urine pH was not altered by a-hANP.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before a-hANP</th>
<th>Before placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>80.7 ± 3.4</td>
<td>81.0 ± 3.4</td>
</tr>
<tr>
<td>Intra-arterial pressure,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0930-1030 hours (mm Hg)</td>
<td>116 ± 4.3</td>
<td>112.5 ± 2.1</td>
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<tr>
<td>24 hr urine sodium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol)*</td>
<td>98 ± 9</td>
<td>97 ± 10</td>
</tr>
<tr>
<td>24 hr urine potassium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol)*</td>
<td>46 ± 3</td>
<td>52 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

a-hANP = α-human atrial natriuretic peptide.

*Measurements in 24-hour collections ending at 0800 hours on the day of a-hANP or placebo injection.
FIGURE 1 A and B. Urine response to the intravenous injection of 100 μg α-human atrial natriuretic peptide (open columns) and placebo (filled columns) in six patients with essential hypertension. The arrow indicates the time of injection. Results are shown as means ± SEM. *p < 0.05, **p < 0.01, t < 0.001, comparing time-matched urine collections on the days of α-human atrial natriuretic peptide and placebo injection.

Arterial Pressure and Heart Rate

From 30-minute mean recordings, there was a significant rise in heart rate after α-hANP administration, which was apparent in the first 30 minutes and was maintained (Figure 2). Arterial pressure was not altered significantly (Figure 2); however, recordings integrated over 60-second intervals revealed a prompt fall in blood pressure and a reciprocal rise in heart rate. Compared with time-matched placebo data, mean arterial pressure had fallen 10.4 ± 3.2 mm Hg 3 minutes after α-hANP injection (p < 0.01) and heart rate was 13.6 ± 2.8 beats/min higher at 4 minutes (p < 0.001). Thereafter, pressure recordings returned steadily to placebo values over 7 minutes.

Hormones

Plasma aldosterone fell within 30 minutes after α-hANP administration to approximately half the time-matched levels (Figure 3). Plasma renin activity and cortisol concentration were not altered (Figure 3). Plasma norepinephrine concentration rose, was 196 pg/ml on average above the matching placebo level 5 minutes after α-hANP administration, then declined over the next 30 minutes (Figure 4). In contrast, epi-
Plasma epinephrine levels fell gradually to a nadir of 48 ± 7 pg/ml 25 minutes after α-hANP administration and returned to preinjection values over the next 60 minutes (Figure 4).

Comparison with Responses in Normotensive Volunteers

The magnitude of the response for some urine components was greater in hypertensive patients than in normotensive subjects studied previously under the same conditions. In the 30 minutes after α-hANP injection, the urinary volume was 405 ± 61 ml greater than that after placebo injection in the hypertensive subjects, but it was only 212 ± 58 ml greater in the normotensive subjects (p < 0.05). The sixfold rise in sodium output over 30 minutes was greater than the fourfold increase measured in the normotensive subjects (p < 0.05). Further, the cumulative excretion of sodium for hypertensive patients exceeded that in the normotensive subjects. For example, in the 90 minutes after α-hANP administration the total output of sodium (compared with that for placebo) was 36 ± 5 mmol in hypertensive subjects and 19.4 ± 4 mmol in the normotensive subjects (Figure 5).

The amount of sodium excreted in the 30 minutes after α-hANP administration correlated positively to the level of intra-arterial pressure measured continuously for 30 minutes immediately before the injection. This finding was true for both hypertensive (r = 0.72) and normotensive subjects (r = 0.78), and the relation persisted when all results were combined (Figure 6).

Short-term changes in arterial pressure and heart rate were similar in the two groups. Over the longer term (30-min mean recordings for 3 hours), the rise in heart rate was almost identical in both groups; however, the normotensive group showed a statistically significant fall in arterial pressure after α-hANP administration while the hypertensive group did not. This difference in response between the two groups was of borderline statistical significance (F = 5.19, df = 1, 8, p = 0.052) in an analysis of variance of differences between α-hANP and placebo in percent change from baseline with two grouping factors (hypertension/normotension and order) and one repeated-measures factor (time).
Discussion

When injected into healthy volunteers, α-hANP is known to have renal, hemodynamic, and hormonal effects.5 The present study shows that the peptide also has clear-cut actions in patients with essential hypertension. A rapid, vigorous output in urine volume, sodium, calcium, magnesium, and phosphorus occurred in all six patients. Arterial pressure fell briefly, whereas the reciprocal rise in heart rate was sustained. Plasma aldosterone concentration fell in the absence of a significant change in renin or cortisol levels, which suggests a direct inhibitory action of α-hANP on the adrenal glomerulosa, as has been shown with atrial peptides in animal studies.10,11 In common with our normotensive volunteers5 the hypertensive subjects exhibited a decline in epinephrine levels after α-hANP administration. This decline may represent an inhibitory effect of the peptide on the adrenal medulla, as was reported from in vitro work.12

Some of the responses in the hypertensive subjects were similar to those reported in normotensive subjects. The time course for the increase in urine indices was much the same in both groups; heart rates rose rapidly and to a similar degree; an initial brief decline in arterial pressure was seen in the two groups; a significant fall in plasma epinephrine concentration was common to both; and neither group showed a change in plasma renin activity or cortisol levels.

There were, however, differences in responses between the groups. First, the output in urine volume, sodium, calcium, and magnesium was greater in the hypertensive subjects despite the fact that they were heavier (by a mean of 10 kg) and therefore received less α-hANP per unit body weight. That the hypertensive subjects received less peptide per unit body weight was confirmed by our finding that immunoreactive α-hANP levels were lower in plasma drawn 5 and 15 minutes after injection (857 ± 51 pmol and 203 ± 4 pmol) compared with time-matched samples in the normotensive subjects (1213 ± 52 pmol and 342 ± 20 pmol; p < 0.05 by t test), although by 30 minutes the levels were similar (136 ± 9 pmol and 128 ± 3 pmol respectively). This radioimmunoassay (unpublished data, 1985) used a specific antiserum supplied by Peninsula Laboratories (Belmont, CA, USA), 125I-α-hANP, and standards of pure α-hANP (Bachem). We did not match the dietary intakes of calcium and magnesium for the two groups, but sodium intake was the same. Further, the excretion rates for these three ions and of urine volume were almost identical in 30-minute collection periods up to the time of α-hANP (and placebo) injection in both groups. That this enhanced urine response might relate to the level of arterial pressure receives support from the strong statistical correlation between preinjection pressures and the magnitude of the natriuresis. In this regard it is interesting to note that the natriuretic response to furosemide (with which atrial peptides have been compared) is by contrast the same or less in hypertensive patients compared with that in normotensive subjects.13-15

Second, the hypertensive subjects had little or no fall in arterial pressure apart from the brief hypotensive response immediately after α-hANP injection. This response contrasts with the small but sustained decline in pressure in the normotensive volunteers previously studied using the same measurement technique.3 Third, hypertensive subjects showed a fall in plasma aldosterone concentration, whereas normotensive subjects did not. However, there was a trend for aldosterone concentration to fall after α-hANP injection in normotensive subjects, and lower and more variable baseline values may have obscured a significant inhibitory effect of α-hANP.

Finally, a brief rise in plasma norepinephrine levels occurred soon after the injection of α-hANP in the hypertensive subjects but not in normotensive subjects. We presume this rise reflects an arterial baroreceptor-mediated increase in sympathetic activity triggered by the sudden fall in arterial pressure. Apart from the fact that the decline in arterial pressure was somewhat faster in the hypertensive group, our studies give little clue as to the reason for this difference. Although it is tempting to ascribe some of these differences to the levels of arterial pressure alone, interpretation must be made with some caution. The hypertensive subjects were older (by a mean of 11 yr) and weighed more (by a mean of 10 kg). They were studied a few weeks after the normotensive subjects when air temperatures were a few degrees warmer. Five of the six patients had previously taken antihypertensive medications. Finally, despite the fact that we used meticulous measurement techniques and standardized conditions of diet and body posture, the number of subjects in each group was not large, and it is possible that our findings are not representative of all hypertensive persons. Further studies are obviously needed.

In conclusion, this study shows that the intravenous injection of 100 μg of α-hANP in subjects with essential hypertension has prominent effects on urine output, heart rate, and plasma hormone levels. Compared with normotensive volunteers, the hypertensive subjects had greater responses in urine volume and urine sodium, calcium, and magnesium output, but a less sustained fall in arterial pressure. Further studies with measurements of plasma α-hANP levels and full dose-response comparisons are needed to clarify the role, if any, of atrial peptides in the pathophysiology of essential hypertension.

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