Prolonged Administration of Human Atrial Natriuretic Peptide in Healthy Men
Reduced Aldosteronotropic Effect of Angiotensin II

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SUMMARY The effect of angiotensin II (5, 10, and 20 ng/kg/min) on blood pressure and on the plasma concentrations of aldosterone was studied in six healthy men with and without the concomitant administration of synthetic human atrial natriuretic peptide given 1) as an i.v. bolus of 25 μg followed by a 6-hour infusion of 25 μg/hr or 2) as an i.v. bolus of 175 μg followed by a 6-hour infusion of 100 μg/hr. The pressor effect of angiotensin II (i.e., the rise of mean blood pressure above individual basal levels) remained unchanged during the administration of both doses of human atrial natriuretic peptide. The angiotensin II-induced rise in plasma concentrations of aldosterone (in terms of absolute values) was reduced by human atrial natriuretic peptide during both trials. The rise in plasma concentrations of aldosterone above individual basal concentrations was also reduced during the administration of human atrial natriuretic peptide, although this effect was only marginal during the low dose experiment. These effects of human atrial natriuretic peptide support the contention that its therapeutic impact in hypertensive patients might be mediated in part by a reduction of high aldosterone concentrations. (Hypertension 8: 1040-1043, 1986)

KEY WORDS • human atrial natriuretic peptide • aldosterone • blood pressure

Atrial natriuretic peptides inhibit aldosterone secretion in vitro and in the conscious rat. Although initial studies in healthy men using human atrial natriuretic peptide (hANP) failed to demonstrate a suppression of aldosterone secretion by hANP, a decrease in plasma aldosterone is indeed seen in healthy men during the continuous infusion of a large dose of hANP. It was therefore of interest to study the effect of hANP on the aldosteronotropic action of angiotensin II in humans.

Subjects and Methods
Nine healthy, nonobese, male volunteers, aged 22 to 35 years, were carefully informed about the aim and the possible risks of the study and gave their written consent to participate. The protocol was approved by the local ethics committee. No medication was permitted for at least 4 weeks before the study. The effect of each dose of hANP was investigated in a group of six volunteers, three of whom participated in both experiments. As described below, each single trial included a control experiment and no comparison of the two groups of subjects (i.e., the 2 doses of hANP) was intended. To induce a positive Na+ balance, all subjects were told to consume their regular diet with 3 g of added salt (NaCl) per day (1 g t.i.d.) for 3 days before each test on an outpatient basis. Hence, mean urinary Na+ excretion rates during the 24 hours preceding the experiments were 205 ± 105 and 193 ± 55 mmol/24 hr, respectively.

On the day of the experiments the subjects fasted from 0000 to 0700. Subsequently, the volunteers remained in the supine position and drank 200 ml of tap water at hourly intervals. Indwelling catheters were inserted in each antecubital vein, one for infusion and the other for blood sampling. An i.v. infusion of 5.0% fructose (6 ml/hr) was started at 0700. Angiotensin II (Hypertensin; CIBA, Basel, Switzerland), diluted in 5.0% fructose, was infused from 0900 to 0945 at increasing doses of 5, 10, and 20 ng/kg/min (3.85, 7.71, and 15.42 pmol/kg/min) for 15 minutes each.

At 1100 the subjects were given hANP (Bissendorf, Federal Republic of Germany) either (Experiment 1)
as an i.v. bolus of 25 μg followed by a 6-hour i.v. infusion of 25 μg/hr (in 30 ml of 5% fructose; 6 ml/hr) or (Experiment 2) as an i.v. bolus of 175 μg followed by a 6-hour i.v. infusion of 100 μg/hr (in 30 ml of 5% fructose; 6 ml/hr). From 1600 to 1645 (i.e., after 5 hours of preexposure to hANP), a second angiotensin II infusion test was performed as already described.

Blood pressure was measured manually every 30 minutes from 0700 to 1700 by the same person using a cuff sphygmomanometer. From 15 minutes before until the end of the two infusions of angiotensin II, blood pressure readings were obtained every 5 minutes. Mean blood pressure was calculated as diastolic blood pressure plus one third of the pulse pressure. Basal blood pressure was defined as the mean of four blood pressure measurements within the 15 minutes preceding the administration of angiotensin II.

Urine collections were obtained before (0700–0900, 0900–1100) and during (1100–1300, 1300–1500, and 1500–1700) the administration of hANP to determine the excretion rates of Na⁺, K⁺, and Cl⁻. Blood samples for the determination of Na⁺, K⁺, and Cl⁻ were obtained before (0900) and after (1700; i.e., +360 minutes) the administration of hANP. Two blood samples for the determination of plasma renin concentration (PRC) and plasma aldosterone concentration (PRA) were drawn before each infusion of angiotensin II. Additional samples for the determination of plasma aldosterone were obtained during and after the infusion of angiotensin II. One sample after each single dose plus two samples during the infusion of the highest dose of angiotensin II (i.e., at 0915, 0930, 0935, 0940, and 0945 and at 1615, 1630, 1635, 1640, and 1645).

Concentrations of Na⁺ and K⁺ in urine and serum were determined by routine biochemical methods (American Monitor Parallel, Indianapolis, IN, USA). The PRC was determined radioimmunologically as reported previously12 and expressed as Goldblatt units (GU) per milliliter. Aldosterone was determined radioimmunologically following extraction by dichloromethane and thin-layer chromatography (cyclohexane/ethyl acetate, 20:80).13 The intra-assay coefficient of variation was 9% (aldosterone) and 8% (PRA). Values are means ± SD.

Data in text, tables, and figures are presented as means ± SD. Student’s t-test (two-tailed) for matched pairs and Bonferroni’s method were used for statistical evaluation.14

### Results

An increase in urine volume and in the excretion rates of Na⁺ was seen during both trials with hANP, whereas excretion rates of K⁺ and endogenous clearance of creatinine remained unchanged (Table 1).

Whereas mean blood pressure readings during Experiment 1 (low dose hANP) were similar before the two infusions of angiotensin II (88 ± 10 and 87 ± 11 mm Hg, respectively), an hANP-induced reduction in mean blood pressure (from 91 ± 12 to 85 ± 9 mm Hg; p<0.05) was seen during Experiment 2 (high dose hANP). Absolute mean blood pressure readings during the infusion of angiotensin II were unchanged by hANP during Experiment 1 but were reduced during Experiment 2 (p<0.01; Figure 1). However, the presor response (i.e., the increments above individual basal levels) to angiotensin II per se was unchanged during both experiments.

Only minor changes were seen in the serum concentrations of Cl⁻ throughout the test periods, and serum concentrations of Na⁺ and K⁺ remained unchanged. Plasma concentrations of renin, as determined before each infusion of angiotensin II, were similar (Table 2).
Mean plasma concentrations of aldosterone, as determined before the infusion of angiotensin II, were slightly reduced by low dose hANP (3.5 ± 1.5 ng/dl; basal, 5.5 ± 2.3 ng/dl; p < 0.05). This difference was less pronounced during Experiment 2, possibly because of a larger interindividual variability (high dose hANP, 5.1 ± 1.9 ng/dl; basal, 8.0 ± 5.2 ng/ml; p > 0.05). Plasma concentrations of aldosterone (Figure 2) were reduced during both infusions of hANP in terms of absolute values (p < 0.01) and in terms of increments above individual basal concentrations (p < 0.01), although the latter effect was only marginal during the low dose infusion of hANP.

Discussion

The availability of hANP* has stimulated investigations of its effects on blood pressure, diuresis, natriuresis, and the plasma concentrations of renin and aldosterone in humans.9•11 In accordance with these reports, in the present study we found that hANP stimulates diuresis and urinary Na+ excretion, but not urinary K+ excretion, in healthy men. Interindividual differences (e.g., in body weight and volume expansion) among healthy, male volunteers may be responsible for the fact that the lower dose of hANP used in the present study exerted a diuretic and natriuretic action while it failed to do so in a previous set of experiments.11 The transient character of hANP-induced diuresis and natriuresis and the fall in mean blood pressure seen during the administration of the larger dose of hANP are, however, in accordance with our previous report.11

The present study was not devised to investigate a possible interference of angiotensin II with hANP-induced diuresis or natriuresis, but rather to evaluate the peptide’s effect on the pressor and the aldosterone-secretory action of angiotensin II. Our results suggest that hANP does not interfere with the angiotensin II–induced rise in blood pressure and that the observed effect on mean blood pressure is due exclusively to a reduction in basal blood pressure. The observation of an unchanged pressor response to angiotensin II in healthy men is in accordance with other reports,15 although contradictory to data obtained in vitro (e.g., in isolated rabbit aortic rings).16

The observed impairment of the angiotensin II–induced rise of aldosterone has been seen after exposure to hANP or to related peptides in various in vitro systems9•15 as well as in the conscious rat17 and may explain the inhibition of aldosterone secretion during atrial stretch.17 This effect was pronounced during the infusion of the larger dose of hANP, but only marginal during the administration of the smaller dose. Increase of endogenous hANP concentrations by volume expansion during Na+ repletion may have blunted the effect of exogenous hANP. However, since only two doses of hANP were employed and different groups of volunteers were studied during these experiments, it is not possible to interpret these data as a dose-response study. It is also of note that the described effect of hANP on angiotensin II–induced aldosterone secretion was present after 5 hours of constant infusion of hANP (i.e., even beyond the compound’s maximum effect on diuresis and natriuresis). Since the volunteers’ fluid balance was clearly negative at this point, their reduced secretion of aldosterone cannot be explained by a suppression of their renin-angiotensin system. In
fact, plasma concentrations of renin were similar at the initiation of the two infusions of angiotensin II. Thus, unlike most diuretics commonly employed in clinical practice, hANP — at least during a 6-hour observation period — induced diuresis and natriuresis without activation of the renin-angiotensin system and in the absence of $K^+$ loss. Suppression by hANP of plasma aldosterone concentrations apparently results from the compound’s direct action on the adrenal cortex, possibly through an inhibition of a common pathway distal to adrenal receptors for various aldosteronotropic stimuli. This apparent direct effect of hANP on the adrenal cortex emphasizes the compound’s potential therapeutic use in hypertension, notably in patients with high prevailing concentrations of aldosterone.

References
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