J. steroid Biochem. Vol. 27, No. 4–6, pp. 935–940, 1987
Printed in Great Britain.

0022-4731/87 $3.00 + 0.00

Proceedings of the VII International Congress on Hormonal Steroids (Madrid, Spain, 1986)

α-h-ANP INJECTION IN NORMALS, LOW RENIN HYPERTENSION AND PRIMARY ALDOSTERONISM

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Summary—Atrial natriuretic peptide, a hormone secreted by the heart, is involved in salt and fluid homeostasis and also exerts an inhibitory effect on aldosterone production *in vitro*. In order to elucidate if this effect is also present in man, 6 normal volunteers, 5 low renin hypertensive patients (LRH) and 7 patients with primary aldosteronism (PA) have received 100 μg of α-h-Anp as bolus i.v. (The decrease in blood pressure was mild and transient in all groups, whereas a marked diuretic effect was observed in all hypertensives even in PA where high levels of endogenous ANP have been found. In normals we observed a significant decrease of plasma aldosterone values while in PA and LRH this effect was not evident. This phenomenon associated with a greater natriuretic effect in LRH and PA, as compared with normals, demonstrates the lack of the correlation between ANP-induced diuresis and aldosterone inhibiting properties.

INTRODUCTION

Atrial natriuretic peptide (ANP), is synthesised, stored and released into the circulation, by atrial myocytes of several species, including man; since it has the properties of an hormone, the heart can be considered as an endocrine organ [1–2]. The physiological role of this hormone has not yet been completely clarified, but it is known that ANP participates in the regulation of sodium-volume balance and has mainly renal and cardiovascular effects. ANP secretion is stimulated by atrial stretch as it occurs in volume expansion, high sodium intake, water immersion or atrial tachycardia among others [2–7]. The action of ANP, when released in circulation, is exerted through its specific receptors located mainly in the kidney (renal glomeruli and vasa recta), on the arterial walls, adrenal glomerulosa cells, posterior pituitary and in several locations within the central and peripheral nervous system.

In fact, there are already many animal studies and increasing evidence in man that ANP has a direct effect on the kidney, leading to an increased GRF (with minimal changes of RBF); that it exerts a systemic depressor effect; an inhibition of aldosterone secretion by the zona glomerulosa, and probably also has a direct inhibition of renin secretion: it also suppresses ADH and Angiotensin II-induced thirst [13–20]. In normal man, acute administration of α-h-ANP causes a rapid diuresis and natriuresis, and a small and transient decrease of blood pressure with minimal change of heart rate [21]. Although it is well established that ANP is a potent inhibitior of basal and stimulated aldosterone secretion *in vitro* [15–17], data concerning its effect on the renin–angiotensin–aldosterone system in man are conflicting [21–25]. Further, a greater diuretic and natriuretic effect and a more evident inhibition of aldosterone has been observed in patients with essential hypertension [22–23]. In primary aldosteronism, a state of chronic volume expansion, endogenous ANP levels are reported to be elevated [26–27]. Furthermore, in this syndrome, the aldosterone hypersecretion is not under normal physiological regulation. We thought, therefore, that this was an interesting model in which the effect of exogenous ANP administration could be studied. Results have been obtained in 7 patients with primary aldosteronism and the responses were compared with those obtained in a group of low renin essential hypertension and in a control group of normal volunteers.

PATIENTS AND METHODS

Six normal men (aged between 24–42 years mean 31 ± 7 years), were studied. They were selected among the students and medical staff of the Department.

Five patients with essential hypertension (aged between 40–64 years, mean 50 ± 8 years), were also studied. Their blood pressure was constantly higher than 160/100 mmHg and there was no evidence of other diseases. They were selected among patients referred to our Center for investigating secondary causes of hypertension, and were found to have some features of mineralocorticoid excess (suppressed upright PRA, transient hypokalemia); however plasma and urinary aldosterone levels were within normal limits. These patients were thus classified as low renin essential hypertensives.

The seven patients with primary aldosteronism had an age range between 17–66 years, (mean 48 ± 16 years). All had hypokalemia and hypertension. The diagnosis of primary aldosteronism was made on the basis of suppressed PRA after upright posture and furosemide and high levels of urinary aldosterone excretion. Five of them were considered to have an aldosterone producing adenoma, by the results of functional tests (plasma aldosterone response to upright posture and to captopril) and by

association of computed tomography and adrenal scintigraphy; when necessary adrenal venography and adrenal aldosterone measurement were performed to confirm the diagnosis. In two patients, no adenoma was demonstrated and they were classified as idiopathic hyperaldosteronism.

In the 5 days before and during 3 days of the study all the subjects were given a controlled diet containing 120 mmol Na^+ and 60 mmol K^+/day. All subjects received no antihypertensive or any other drugs for at least 15 days prior to the study. Each subject served as his own control. On the test day, the patients were allowed to stand between 0700 and 0730 h to void the bladder; thereafter they were placed in a comfortable sitting position. They were fasting from the evening before, and were allowed to drink only one glass of water at 0730 h. Subsequently, a plastic cannule was inserted in a antecubital vein and a slow infusion of 5% of glucose in water was administered for a 30 min equilibration period.

At 0800 a.m., on the first and third day of the study subjects were randomly allocated for an i.v. administration of a bolus of 100 μg of α-h-ANP 1–28 (Bissendorf GmBh, Wedemark, F.R.G.) diluted in 10 ml of saline or for an i.v. bolus of 10 ml of saline. Blood pressure and heart rate were continuously monitored by an automatic device (Dinamap) during the half hour before, and for the 2 h after, the injection of ANP or placebo. Blood samples for determination of serum sodium, potassium, creatinine, plasma renin activity (PRA), aldosterone and cortisol were obtained prior to and then at 0, 5, 10, 15, 30, 45, 60, 90 and 120 min after ANP or placebo injection. Urinary collections were obtained in three daily fractions (0800–1200, 1200–2000, 2000–0800); Sodium, potassium, creatinine and aldosterone were measured in each urinary fraction. Plasma and urinary aldosterone were measured by radioimmunoassay with a kit (Aldo K Sorin Italy). Plasma cortisol was measured by radioimmunoassay (Diagnostic Products U.S.A.). PRA was determined by the method of Stockigt *et al.*[28]. Serum and urinary electrolytes were measured by a flame photometry. Two-way analysis of variance (Anova) and Student' *t*-test were used for statistical evaluation. The results were expressed as Mean ± SE.

RESULTS

Blood pressure and heart rate

Control BP values before the ANP infusion were 137 ± 7/87 ± 3 mmHg in normal volunteers; 167 ± 8/100 ± 6 mmHg in the LRH and 185 ± 7/105 ± 6 mmHg in the patients with primary aldosteronism. The mean decrease of BP was similar in the three groups studied, being respectively 15 ± 3.5/7 ± 3.1 mmHg in normals, 14 ± 2.3/9 ± 3.4 in LRH and 15 ± 5.5/6 ± 2.1 mmHg in primary aldosteronism (Fig. 1). Thus the percent maximal change at the time of maximal decrement was greater in normotensives (10.9% of systolic and 8.1% of diastolic BP) than in hypertensives (8.9 and 8.1% of systolic and 8.5 and 5.7% of diastolic BP respectively), but it was earlier in hypertensives than in normotensives. However, these changes did not achieve statistical significance either in comparison with the pre-ANP values or with the values obtained during placebo. The heart rate (HR) was only slightly but not significantly increased in normals whereas no changes of HR were noticed in hypertensives subjects (Fig. 1).

Serum electrolytes

Serum sodium and potassium were respectively 140 ± 2 and 4.1 ± 0.1 mEq/l in normals, 140 ± 1 and 4.1 ± 0.2 in LRH and 143 ± 3 and 3.3 ± 0.2 in pri-

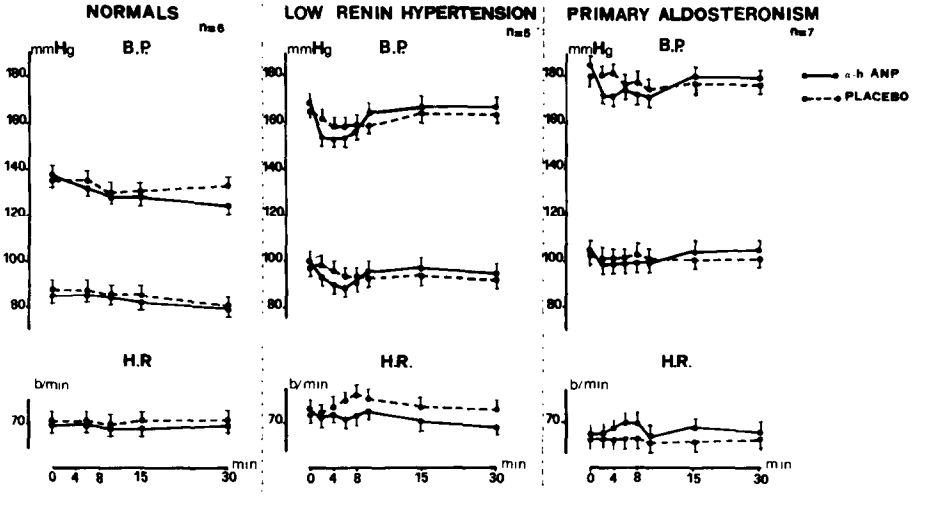

Fig. 1. α-h-ANP 100 μg i.v. as a bolus, caused a mild decrease of blood pressure (BP) in normals, LRH and PA patients. The decrease was earlier in hypertensives patients of both groups. There was no effect on heart rate (HR).

mary aldosteronism. All these values remained unchanged after ANP administration.

Urinary fractions

The first 4 h urinary fractions (0800–1200) collected on the control day were respectively 228 ± 25, 255 ± 30 and 330 ± 22 ml in the three groups. On the day of ANP administration, only a minor increment to 251 ± 56 ml was seen in normals, whereas it was much greater in LRH (493 ± 79 ml) and in primary aldosteronism [505 ± 66] (Fig. 2).

Urinary sodium

The 4 h urinary sodium excretion on the control day was similar in the three groups, after ANP administration there was almost no increment of urinary sodium in the normal subjects, whereas the natriuresis was doubled in the LRH and enhanced by 41.2% in the primary aldosteronism group (Fig. 2).

Urinary potassium

Again, urinary potassium was similar in the 4 h specimens of three groups on the control day, but in this case no major changes were induced by ANP (Fig. 2).

Hormonal data

Plasma renin activity (PRA). The baseline levels of PRA were 4.2 ± 0.8 ng/ml/3h in the normals

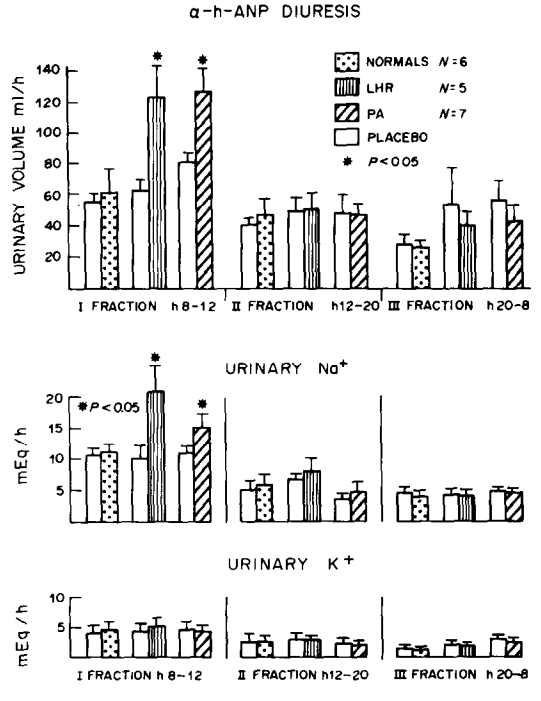

Fig. 2. Effects of ANP on diuresis and urinary electrolytes. The changes of urinary volume and sodium were significant in LRH and PA. Minimal changes was observed in normal subjects. Urinary potassium remained unchanged in all groups.

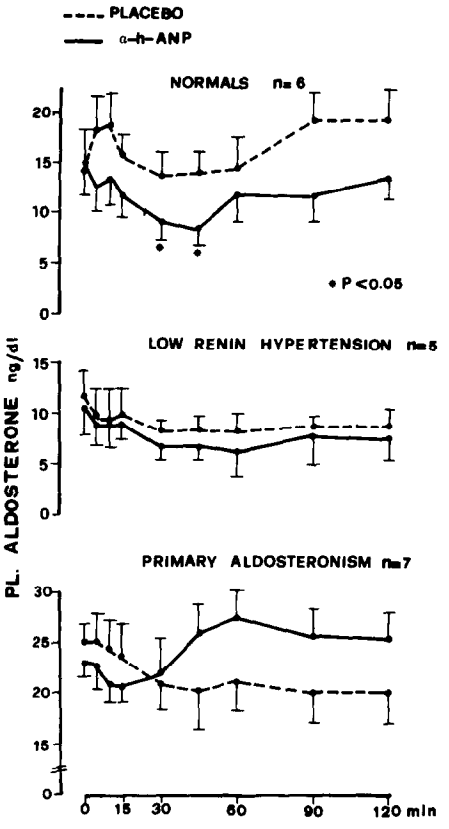

Fig. 3. α-h-ANP 100 μg as a bolus, was able to slightly decrease PRA in normals subjects. No variations were observed on the already suppressed PRA in both hypertensive groups.

before the ANP infusion: after ANP administration there was a decrease of mean values to 3.0 ± 0.4 ng/ml/3h at 30–45 min. The changes were not statistically significant. However on the control day there were almost no changes of PRA, ranging from 3.9 ± 0.7 to 3.5 ± 0.5 ng/ml/3h.

In the two groups with low renin hypertension, ANP did not induce any detectable changes of the already suppressed values (Fig. 3).

Plasma aldosterone

In normal volunteers, baseline plasma aldosterone (in sitting position) was 15.0 ± 3.1 ng/dl. The minimal mean value reached after ANP infusion was 8.9 ± 1.4 ng/dl at 45 min. This change was significant both in comparison of the pre-ANP levels and of the levels measured on the control day. No major changes were seen on the control day.

In the LRH baseline aldosterone values were lower than normal (6.4 ± 1.2 ng/dl), and no changes were induced by ANP administration.

In the primary aldosteronism group, mean baseline plasma aldosterone levels was 23 ± 4.4 ng/dl, after ANP, mean aldosterone values remained almost unchanged (21 ± 3.0 ng/dl) at 30 min (Fig. 4).

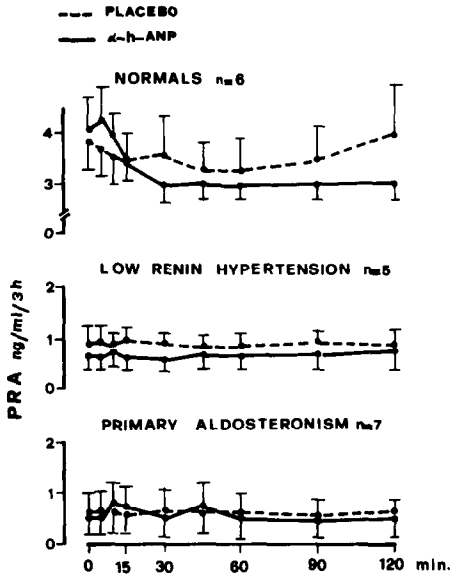

Fig. 4. Plasma aldosterone was significantly decreased at 45 min by α-h-ANP (100 μg as a bolus i.v.) in normals. This effect was not seen either in LRH patients and in PA patients.

Plasma cortisol

In all three groups, plasma cortisol tended to decrease during the two hours of the study, both on the control day and on the day of the test. The changes were not significant. It is likely that this trend was due to the spontaneous circadian variation of cortisol (Fig. 5).

DISCUSSION

From our data it appears that ANP when injected as a bolus had a mild and transient effect on blood pressure, which was similar in the normotensive and in the hypertensive patients. However, when expressed as percent changes this decrement was greater in the normotensive controls. Similarly, heart rate was slightly increased only in this latter group indicating a mild activation of arterial baroreceptors. Although plasma catecholamines were not measured in the present study, it has previously been shown that an increase of norephinephrine may be induced by ANP infusion of humans [23]; this is probably a consequence of the hypotensive effect of ANP. In all likelihood the hypotensive effect of ANP is probably due to its vasorelaxing properties through specific receptors on the vascular walls [9], since a decrease in blood pressure was obtained in normal volunteers in whom almost no diuretic effect was seen; however, it is likely that acute volume and sodium depletion could play an additional role on the blood pressure reduction induced by ANP.

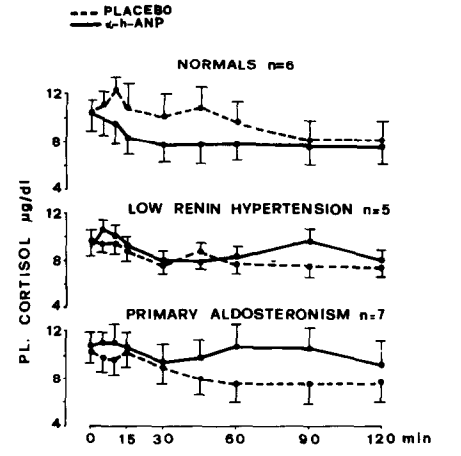

Fig. 5. Plasma cortisol was not modified by the ANP 100 μg i.v. injection, in all three groups.

The prominent effects of ANP are those exerted on the kidney, although it seems that its diuretic and natriuretic effect is very much dependent on the status of the fluid and sodium balance. It has been shown that a greater natriuresis is induced by ANP in a sodium replete man in comparison with the subjects studied after salt restriction [29]. In fact, in our normal subjects, almost minimal renal effect of the ANP bolus was seen; it must be kept in mind that they were not hydrated before the study. Furthermore, it is likely that the 4-h urine collection could have masked small changes induced by the bolus injection of ANP. It was of great interest that, with the same protocol, a clearcut increase of urinary output as well as of natriuresis was obtained in both low renin hypertensives and in primary aldosteronism patients. It is quite possible that the enhanced natriuretic effect of ANP in these clinical conditions could be due to a status of volume expansion and increased exchangeable sodium. However, this has not been uniformly demonstrated in LRH patients nor even in patients with idiopathic aldosteronism. Since an increased diuresis and natriuresis has also been obtained by other investigators in patients with essential hypertension, independently of their plasma renin values [22–23], it is likely that this phenomenon is mostly due to the increased renal perfusion pressure which is characteristic of all hypertensives. Furthermore, our data demonstrate that in cases of chronic volume expansion, such as in primary aldosteronism in whom endogenous levels of ANP have been found to be elevated, a further increase of this level as induced by exogenous administration, is able to exert a greater effect at the renal level. Thus, it seems that no down-regulation of the renal receptors of ANP is induced by a chronically high level of this hormone. Similar data have been recently obtained in normal subjects after high salt intake [29].

The effects of ANP on the renin–angiotensin system were not very apparent; only in the normal volunteers a trend towards a decrease, although not statistically significant, was seen. A significant decrease of renin values after ANP infusion was

reported either in normals and heart failure patients [25]. No changes on PRA were seen by Richards *et al.*[21] after bolus ANP injection, and an increase of PRA was seen by others [23, 29] after ANP prolonged infusion. More recently, a significant decrease of both PRA and Angiotensin II was described in subjects on low salt diet [30]. Thus, it appears that only when baseline PRA is stimulated, is it possible to confirm the direct ANP inhibitory activity which has previously been described by Mack in the dog [31]. In the same experimental conditions (upright posture or low salt diet) even the fall in the plasma aldosterone level, was more evident, suggesting a possible relation between the decrease of PRA and that of aldosterone. It has to be stressed that no changes of aldosterone were found in our two groups with PRA values already suppressed before ANP infusion. However, it is well demonstrated that a peculiar characteristic of ANP is that of being a direct inhibition of aldosterone secretion by glomerulosa cells [15–17]. It is likely that this is the primary mechanism which is responsible for the fall of aldosterone even in situations when PRA remains unchanged or even increases.

The lack of changes of aldosterone in patients with low renin essential hypertension could in fact be simply due to the low baseline aldosterone levels. However, also in primary aldosteronism no effect of ANP on plasma aldosterone levels was seen. An alternative explanation for both cases could be based on the acute way of ANP administration possibly being inadequate for maintaining plasma levels high enough to be effective on the adrenals.

It must be recalled that Glaz and others have shown that a continuous infusion of ANP in a case of Conn's syndrome was able to decrease aldosterone or 18-OH corticosterone levels [32]. We are at present investigating this possibility, in a greater group of patients but our preliminary results seem to confirm our previous negative data. It is of interest that *in vitro* studies of Higuchi *et al.* showed no effect of ANP on aldosterone level and cGMP release from aldosteronoma cells in culture [33]. These cells lacked also ANP receptors. Taken together, our data seem to indicate that the inhibitory effect of ANP on the RAAS is not of fundamental importance for the display of its natriuretic effect, but rather a compensatory phenomenon which should blunt the RAAS reactions to the primary renal effect.

REFERENCES

1. De Bold A. J., Borenstein H. B., Vereas A. T. and Sonnenberg H.: A rapid and potent natriuretic response to intrevenous injection of atrial myocardial extract in rat. *Life Sci.* **28** (1981) 89–94.
2. Cantin M. and Genest J.: The heart and the atrial natriuretic factor. *Endocr. Rev.* **6** (1985) 107–127.
3. Needleman P. and Greenwald J. E.: Atriopeptin: a cardiac hormone intimately involved in fluid electrolyte, and blood-pressure homeostasis. *N. Engl. J. Med.* **314** (1986) 828–834.
4. Hasegawa K., Matsushita Y., Inoue T., Morii H., Ishibashi M. and Yamaji T.: Plasma levels of atrial natriuretic peptide in patients with chronic renal failure. *J. clin. Endocr. Metab.* **63** (1986) 819–822.
5. Sagnella G. A., Shore A. C., Markandu N. D. and MacGregor G. A.: Effects of changes in dietary sodium intake and saline infusion on immunoreactive atrial natriuretic peptide in human plasma. *Lancet* **30** (1985) 1208–1210.
6. Anderson J. V., N. D. Millar, O'Hare J. P., Mackenzie J. C., Corrall R. J. M. and Bloom S. R.: Atrial natriuretic peptide: physiological release associated with natriuresis during water immersion in man. *Clin. Sci.* **71** (1986) 319–322.
7. Yamaji T., Ishibashi M., Nakaoka H., Imataka K., Amano M. and Fujii J.: Possible role for atrial natriuretic peptide in polyuria associated with paroxysmal atrial arrhythmias. *Lancet* **1** (1985) 1211.
8. Murphy M. M., McLaughlin L. L., Michener M. L. and Needleman P.: Autoradiographic localization of atriopeptin III receptors in rat kidney. *Eur. J. Pharmac.* **11** (1985) 291–292.
9. Hirata Y., Tomita M., Yoshimi H. and Ikeda M.: Specific receptors for atrial natriuretic factor (ANF) in cultured vascular smooth muscle cells of rat aorta. *Biochem. biophys. Res. Comm.* **125** (1984) 562–568.
10. Anad-Scrivastava M. B., Cantin M. and Genest J.: Inhibition of pituitary adenylate cyclase by atrial natriuretic factor. *Life Sci.* **36** (1986) 1873–879.
11. Saper C. B., Standaert D. G., Currie M. G., Schwartz D., Geller D. M. and Needleman P.: Atriopetin-immunoreactive neurons in the brain and presence in cardiovascular regulatory areas. *Science* **227** (1985) 1047–1049.
12. Debinsky W., Gutkowska J., Kuchel O., Racz K., Buu N. T., Cantin M. and Genest J.: ANF-like peptides in the peripheral autonomic nervous system. *Biochem. biophys. Res. Commun.* **134** (1985) 279–284.
13. Hintze T. H., Currie M. G. and Needleman P.: Atriopeptins: renal specific vasodilatators in conscious dogs. *Am. J. Physiol.* **248** (1985) H587–591.
14. Beasley D. and Malvin R. L.: Atrial extracts increase glomerular filtration rate *in vivo*. *Am. J. Physiol.* **284** (1985) f24–f30.
15. Atarashi K., Murlow P. J., Franco-Saenz R., Snajdar R. and Rapp J., Inhibition of aldosterone production by an atrial extract. *Science* **22** (1984) 992–994.
16. Goodfriend T. L., Elliot M. E. and Atlas S. A.: Actions of synthetic atrial natriuretic factors on bovine adrenal glomerulosa. *Life Sci.* **35** (1984) 1675–1682.
17. Chartier L., Shiffrin E. L., Thibault G. and Garcia R.: Atrial natriuretic factor inhibits the effect of Angiotensin II, ACTH and potassium on aldosterone secretion *in vitro* and angiotensin II-induced steroidogenesis *in vivo*. *Endocrinology* **115** (1984) 2026–2028.
18. Obana K., Naruse M. and Naruse K.: Synthetic rat atrial natriuretic factor inhibits *in vitro* and *in vivo* renin secretion in rats. *Endocrinology* **117** (1985) 1282–1284.
19. Samson W. K.: Atrial natriuretic factor inhibits dehydration and hemorrhage induced vasopressin release. *Neuroendocrinology* **40** (1985) 277–279.
20. Nakamura M., Katsuura G., Nakao K. and Imura H.: Antidipsogenic action of alpha-human atrial natriuretic polypeptide administered intracerebroventricularly in rats. *Neurosci. Lett.* **58** (1985) 1–6.
21. Richards A. M., Nicholis M. G., Ikram H., Webster M. W. I., Yandle T. G. and Espiner E. A.: Renal, heamodynamic, and hormonal effects of human alpha atrial natriuretic peptides in healthy volunteers. *Lancet* **i** (1985) 545–549.
22. Richards A. M., Nicholls M. G., Espiner E. A., Ikram

H., Yandle T. G., Joyce S. T. and Cullens M. M.: Effects of alpha-human atrial natriuretic peptide in essential hypertension. *Hypertension* **7** (1985) 812–817.

23. Weidmann P., Gnadinger P. M., Ziswiler H. R., Shaw S., Bachmann C., Rascher W., Uelinger D. E., Hsaler L. and Reubi F. C.: Cardiovascular, endocrine and renal effects of atrial natriuretic peptide in man. *J. Hypertens.* **4** (1984) Suppl. 2, S77–S74.
24. Weidmann P., Hasler L., Gnadiger M., Lang R. E., Uehlinger D. E., Shaw S. and Rascher W. and Reubi F. C.: Blood levels and renal effects of atrial natriuretic peptide in normal man. *J. clin. Invest.* **77** (1986) 734–742.
25. Cody R. J., Atlas S. A., Laragh J. H., Kubo S. H., Covit A. B., Ryman K. S., Shaknovich A., Pondolfino K., Clark M., Camargo M. J. F., Scarborough R. M. and Lewicki J. A.: Atrial natriuretic factor in normal subjects and heart failure patients: plasma levels and renal, hormonal and heamodynamic responses to peptide infusion. *J. clin. Invest.* **78** (1986) 1362–1374.
26. Tunny T. J., Higgins B. A. and Gordon R. D.: Plasma levels of atrial natriuretic peptide in man in primary aldosteronism, in Gordon's syndrome and in Bartter's syndrome. *Clin. exp. Pharmac. Physiol.* **13** (1986) 341–346.
27. Yamaji T., Ishibashi M., Sekihara H., Takaku F., Nakaoka H. and Fujii J.: Plasma levels of atrial natriuretic peptide in primary aldosteronism and essential hypertension. *J. clin. Endocr. Metab.* **63** (1986) 815–818.
28. Stockigt J. R., Collins R. D. and Biglieri E. G.: Determination of plasma renin concentration by angiotensin I immunoassay: diagnostic import of precise measurement of subnormal renin in hyperaldosteronism. *Circ. Res.* **28** (1971) Suppl. 2 175–187.
29. Wedmann P., Hellmueller B., Uehlinger D. E., Gnaedinger M. P., Hasler L., Shaw S. and Bachmann C.: Plasma levels of atrial natriuretic peptide during different sodium intakes in man. *J. clin. Endocr. Metab.* **62** (1986) 1027–36.
30. Cuneo R. C., Espiner E., Nicholls M. G., Yandle T. G., Joyce S. L. and Gilchrist N. L.: Renal, hemodynamic and hormonal responses to atrial natriuretic peptide infusions in normal man and effect of sodium intake. *J. clin. Endocr. Metab.* **63** (1986) 946–953.
31. Mack T., Marion D. N., Camargo M. J. F., Kleinert H. D., Laragh J. D., Vaughan E. D. Jr and Atlas S. A.: Effects of auricolin (atrial natriuretic factor) on blood pressure, renal function and the renin–aldosterone system in dogs. *Am. J. Med.* **77** (1984) 1069–75.
32. Glaz E., Kiss R., Racz K., Varga I., Futo L. and Vecsei P.: Hypotensive effect of human natriuretic peptide in patients with primary aldosteronism. *Proceedings of 11th Scientific Meeting of the International Society of Hypertension*, Heidelberg, August 31–September 6, (1986) Abstr. no. 1118.
33. Higuchi K., Nawata H., Kato K-I., Ibayashi H., and Matsuo H.: Lack of inhibitory effect of alpha-human atrial natriuretic polypeptide on aldosteronogenesis in aldosterone producing adenoma. *J. clin. Endocr. Metab.* **63** (1986) 192–196.