NOTE

Changes in the Plasma and Urine α Human Atrial Natriuretic Peptide (α hANP) Concentration in Patients with Thyroid Disorders

YOSHINOBU SUZUKI, HITOSHI SUZUKI, RYUSUKE OHTAKE,
HIROBUMI KOBORI, TOKIHIKO TSUCHIYA, YOSHIHISA HASHIGAMI
AND SHIN-ICHI SHIMODA

*Department of Endocrinology, Internal Medicine,
Dokkyo University School of Medicine,
Mibu, Tochigi 321-02, Japan*

Abstract

In order to assess the possible involvement of thyroid hormone in α human atrial natriuretic peptide (α hANP), we investigated the plasma and urine ANP concentration in patients with primary hyperthyroidism and hypothyroidism. Plasma and urine were extracted through Sep-Pak C₁₈ cartridges and the urine ANP concentration was corrected by urine creatinine (cre. mg/dl) and expressed as fmol/mg·cre.. The plasma ANP concentration in patients with untreated hyperthyroidism (32.3 ± 7.0 fmol/ml; $n=22$) was higher than in normal subjects ($p < 0.01$ vs control; 6.2 ± 0.7 fmol/ml). After restoration to euthyroidism, the plasma ANP concentration (patients with treated hyperthyroidism) fell to normal (8.9 ± 1.9 fmol/ml). The plasma ANP concentration in patients with untreated hypothyroidism (14.1 ± 3.0 fmol/ml; $n=7$) was higher than normal, but in two of them there was mild renal dysfunction and an incomplete right bundle branch block in the electrocardiogram. It was possible that these factors contributed to the observed increase in plasma ANP. However, a significant positive correlation was found between plasma ANP and free thyroxine ($n=40$, $r=0.449$; $p < 0.01$) and free triiodothyronine ($n=40$, $r=0.546$; $p < 0.01$). The urine ANP concentration in patients with untreated hyperthyroidism was markedly higher than in normal subjects ($p < 0.01$), but in untreated hypothyroidism not significantly different from normal.

It is well-known that thyroid hormone, which has positive inotropic and chronotropic actions, influences the cardiovascular

system. The heart rate, stroke volume and cardiac output are increased in hyperthyroidism, and inversely all of the parameters are reduced in hypothyroidism (Buccino *et al.*, 1967; Amidi *et al.*, 1968; Grossman *et al.*, 1971; Sefidper *et al.*, 1971).

It was reported that a rapid and potent natriuretic response to intravenous injection

Received September 30, 1987

Correspondence to YOSHINOBU SUZUKI, M.D., Dept. of Endocrinology, Internal Medicine, School of Medicine Dokkyo University, Mibu, Tochigi 321-02, Japan

of atrial myocardial extracts occurs in rats (De Bold *et al.*, 1981) and later the complete amino acid sequence of atrial natriuretic peptide (ANP) was determined (Kangawa *et al.*, 1984).

ANP has potent natriuretic, diuretic and vasorelaxant actions and is closely related with the circulating blood volume (Lang *et al.*, 1985; Anderson *et al.*, 1986 b), and the plasma ANP concentration is increased in various conditions which caused blood or extracellular fluid to increase, i.e. congestive heart failure (Burnett *et al.*, 1986; Tikkanen *et al.*, 1985), chronic renal failure (Anderson *et al.*, 1986 a; Rascher *et al.*, 1985; Suzuki *et al.*, 1987) and pregnancy (Cusson *et al.*, 1985).

In this study, we used a radioimmunoassay for α hANP constituted with labeled α hANP and rabbit anti- α ANP antiserum obtained from a commercial source, and investigated the plasma and urine ANP concentrations in patients with thyroid disorders to see whether any significant changes could be found in these conditions.

Materials and Methods

Subjects

Twenty-two patients with untreated hyperthyroidism (18 women and 4 men, mean age 32.6 ± 1.3 yrs., range 13–67 yrs.), ten patients with treated hyperthyroidism (8 women and 2 men, mean age 26.5 ± 3.8 yrs., range 11–47 yrs.), seven patients with untreated primary hypothyroidism (5 women and 2 men, mean age 45.9 ± 9.3 yrs., range 11–79 yrs.), four with treated hypothyroidism (4 women, 30–43 yrs., mean age 38.3 ± 4.0 yrs.) and fifty-one normal subjects (26 women and 25 men, mean age 32.8 ± 1.3 yrs., range 16–60 yrs.) were studied. None of the normal subjects showed evidence of any disease and none had taken drugs. The diagnosis of thyroid disorders was based on the clinical assessment and confirmed by radioimmunoassay of serum total thyroxine (TT₄), total triiodothyronine (TT₃), free thyroxine (fT₄), free triiodothyronine (fT₃) and TSH. TT₄, TT₃ and TSH were measured with kits purchased from Dainabot RI Lab.

Tokyo Japan. FT₄ and fT₃ were measured with kits purchased from Travenol Co. Ltd. and Amersham International plc., respectively. The patients with hyper- and hypothyroidism were treated with an antithyroid drug (propylthiouracil or methimazole) or levo-thyroxine Na. The mean duration of treatment was 161.6 ± 57.1 days and 2.4 ± 0.8 yrs, respectively.

Preparation of Samples

Blood samples (usually 10 ml) were collected in plastic tubes containing aprotinine (400 KIU/ml) and EDTA (1 mg/ml) from subjects in a resting, supine position, and urine samples were obtained from morning partial urine. The patients were allowed to take sodium and water freely. The plasma was immediately separated by centrifugation (750 xg) for 20 min at 4°C. ANP was extracted from plasma or urine with Sep-Pak C₁₈ cartridges (Waters Associates Milford MA, recovery $71.8 \pm 0.6\%$) according to the method described in our previous report (Suzuki *et al.*, 1987).

Radioimmunoassay Procedure

The immunoreactive ANP (IR-ANP) in plasma and urine was determined with (3-[¹²⁵I]iodotyrosol ²⁸) α hANP (Amersham International plc.) as tracer and rabbit anti- α ANP antiserum (Cat No. RAS8798 and Lot No. 009325, Peninsula Lab. Inc.). Details of the assay are given elsewhere (Suzuki *et al.*, 1987). The coefficients of variation in the intra- and interassay with the same reagents for 7 and 5 replicates of the median concentration of ANP were 10.9 and 10.6%, respectively.

Reverse phase high performance liquid chromatography (HPLC)

IR-ANP was characterised by reverse phase HPLC. HPLC was performed on a μ Bondashere 5 μ C₁₈-100A column (3.9 $\times$ 150 mm, Waters Japan, Co.) under stepwise isocratic elution at 10, 35 and 40% acetonitrile (ACN)/water containing 0.1% trifluoroacetic anhydride (TFA). The HPLC system included a pump (LC-6A, Shimadzu Corporation Kyoto, Japan), column oven (CTO-6A, Shimadzu Corp. Kyoto, Japan) and injector (7125, Rhepdyne Cotaro, California, USA). Plasma and urine extracts obtained from hyperthyroid patients were applied to the column at a flow rate of 1 ml/min and 2 ml fractions were collected. These fractions were evaporated to dryness. The dry residue was reconstituted

in 200 μ l of assay buffer and assayed.

Calculation

Statistical analysis was performed by Student's *t*-test and linear regression analysis. Results were expressed as the mean $\pm$ SEM of molar equivalents of synthetic α hANP. The plasma IR-ANP concentration was expressed as femtomolars per ml. The urine IR-ANP concentration was corrected for urine creatinine (mg/dl, cre.) and expressed as femtomolars per mg creatinine (fmol/mg cre.).

Results

Plasma and Urine IR-ANP concentrations in thyroid disorders

The plasma IR-ANP concentration and/or thyroid function in normal subjects and patients with hyper- and hypothyroidism are shown in Fig. 1 and Table 1, respectively. In normal subjects, the plasma IR-ANP concentration ranged from 2.5–16.2 fmol/ml (mean value of 6.2 ± 0.7 fmol/ml) and the urine IR-ANP concentration ranged from 2.6–23.0 fmol/mg cre. (mean value of 8.9 ± 0.8 fmol/mg cre.). The plasma IR-ANP concentration in patients with untreated hyperthyroidism was 32.3 ± 7.0 fmol/ml and significantly higher than in normal subjects ($p < 0.01$). A particularly high

Fig. 1. Plasma ANP concentration in patients with hyper or hypothyroidism before and after treatment.

value of over 100 fmol/ml was observed in two cases. None of them had arrhythmias, congestive heart failure or cardiac effusion, though one had mild cardiomegaly (cardiothoracic ratio = 58.6%) and the other had very high cardiac output (C.O. = 10.57 l/min,

Table 1. Thyroid function and plasma ANP concentration in patients with thyroid disorders.

	Untreated Hyper- thyroidism (n=22)	Treated Hyper- thyroidism (n=10)	Untreated Hypo- thyroidism (n=7)	Treated Hypo- thyroidism (n=4)
Serum fT ₄ (ng/dl)	4.5 ± 0.2	2.0 ± 0.3	0.3 ± 0.1	0.9 ± 0.1
Serum fT ₃ (pg/ml)	16.2 ± 1.5	7.2 ± 1.0	2.0 ± 0.6	3.0 ± 1.5
Serum TT ₄ (μ g/dl)	19.6 ± 1.0	10.8 ± 1.5	2.0 ± 0.5	5.7 ± 0.4
Serum TT ₃ (ng/ml)	3.5 ± 0.3	2.1 ± 0.3	0.7 ± 0.1	1.1 ± 0.2
Serum TSH (μ U/ml)	—	—	136.2 ± 40.9	6.9 ± 2.1
Plasma ANP (fmol/ml)	32.3 ± 7.0	8.9 ± 1.9	14.1 ± 3.0	6.5 ± 0.5

All values are expressed as mean $\pm$ SEM.

Numbers in parenthesis denote cases studied.

Normal ranges: fT₄: 0.8–2.4 (ng/dl), fT₃: 2.0–6.0 (pg/ml),

TT₄: 5.0–13.0 (μ g/dl), TT₃: 0.8–1.8 (ng/ml),

TSH: <9 (μ U/ml).

cardiac index = 7.1 l/min/m^2). However, this high IR-ANP concentration observed in hyperthyroidism returned to the normal range ($8.9 \pm 1.9 \text{ fmol/ml}$) after treatment ($p < 0.05$ vs untreated hyperthyroidism). The plasma IR-ANP concentration in untreated hypothyroidism was also significantly higher than normal ($14.1 \pm 3.0 \text{ fmol/ml}$, $p < 0.01$). Among three hypothyroid patients with high circulating ANP, two showed other abnormalities: one case was very old and had mild renal dysfunction, and the other case had incomplete right bundle branch block (RBBB) on electrocardiogram (ECG). The plasma ANP concentration ($9.9 \pm 1.8 \text{ fmol/ml}$) in untreated hypothyroidism, except for two cases, was higher than normal ($p < 0.05$). The plasma ANP concentration in treated hypothyroidism was $6.5 \pm 0.5 \text{ fmol/ml}$ and not significantly different from normal. The correlation between the serum

thyroid hormone and plasma IR-ANP concentration was assessed in 40 samples from 43 patients with thyroid disorders, where both thyroid hormone and ANP could be measured simultaneously. A significant positive correlation was found between IR-ANP and fT_4 ($n = 40$, $r = 0.499$, $p < 0.01$) and fT_3 ($n = 40$, $r = 0.546$, $p < 0.01$) and the correlation coefficient was greatly improved if two hypothyroid patients with complications were deleted, although no significant correlation between plasma ANP and thyroid hormone could be observed in these patients when the calculation was performed for each group separately. The circulating ANP and fT_4 concentrations in 9 out of 22 hyperthyroid patients, whose circulating hormones could be measured several times during the treatment, are plotted in Fig. 2. These concentrations decreased simultaneously after the treatment.

Fig. 2. Plasma ANP and serum free T_4 concentration in pre- and post treatment of nine patients with hyperthyroidism.

Fig. 3 (a). Fractionation of IR-ANP in plasma extracts by reverse phase HPLC. A stepwise isocratic elution with 10, 35 and 40% of acetonitrile (ACN)/water with 0.1% TFA (see Materials and Methods) was used (---) at a flow rate of 1 ml/min.

Fig. 3 (b). Fractionation of IR-ANP in urine extracts by reverse phase HPLC. A stepwise isocratic elution with 10, 35 and 40% of acetonitrile (ACN)/water with 0.1% TFA (see Materials and Methods) was used (---) at a flow rate of 1 ml/min.

Urine IR-ANP concentrations in untreated hyper- and hypothyroidism were 15.6 ± 2.8 and 9.8 ± 4.2 fmol/ml-cre., respectively. The level in hyperthyroidism was significantly higher than normal ($p < 0.01$) though there was not a significant difference

between hypothyroid and normal urine ANP.

Reverse phase HPLC

Plasma and urine extracts from patients with hyperthyroidism were subjected to reverse phase HPLC and the chromatographic profiles are shown in Fig. 3 (a) and (b). The main immunoreactive peaks in extracts eluted at 35% ACN/water with 0.1% TFA and were identical to those of pure synthetic α hANP.

Discussion

In order to assess the involvement of thyroid hormone in ANP, we performed α hANP measurement in plasma and urine in hyperthyroidism and hypothyroidism. The plasma IR-ANP concentration was markedly increased in untreated hyperthyroidism, which returned to normal after treatment with an anti-thyroid drug. Kohno *et al.* (1986 (a), 1986 (b)) reported that plasma ANP was increased in experimental hyperthyroid rats and patients with thyrotoxicosis and decreased by successful treatment with methimazole. These results were in accordance with those of the present study. Argentin *et al.* (1987) reported thyroid hormone (T_3) stimulated rat pro-natriodilation, precursor of ANP, mRNA synthesis in primary culture of rat neonatal cardiocytes. Their results may favor a direct thyroid hormone action on ANP synthesis and secretion from heart. However, because of the marked hemodynamic change in hyperthyroid states, we could not conclude whether the high plasma IR-ANP concentration observed in patients with hyperthyroidism might be due to the circulatory change or

to the direct stimulation of thyroid hormone.

In hypothyroid patients, the plasma IR-ANP concentration was significantly higher than normal and this result conflicted with the report of Zimmerman *et al.* (1987). In this study, two of 7 untreated hypothyroid patients showed other mild pathological symptoms; one patient was very old and had mild renal dysfunction and the other had incomplete RBBB on ECG. Furthermore the mean age of the untreated hypothyroid patients was the highest among the groups. High plasma ANP had been reported to be present in chronic renal failure (Anderson *et al.*, 1986 (a), Rascher *et al.*, 1985, Suzuki *et al.*, 1987) and in healthy elderly men (Ohashi *et al.*, 1987). These factors might have modulated circulating ANP in the hypothyroid patients in this study.

ANP was thought to be mainly metabolized by kidney (Tang *et al.*, 1984) and in fact ANP was demonstrated to be present in urine (Marumo *et al.*, 1986, Suzuki *et al.*, 1987), but the physiological significance of urine ANP is still unknown. Urine ANP in hypothyroidism was not markedly different from normal, but in hyperthyroidism it was seen to increase markedly in this study. In the present study we showed that not only circulating ANP but also the urine ANP concentration was markedly increased in patients with hyperthyroidism. This result might provide additional evidence of increased ANP production and secretion in hyperthyroidism. However, no significant correlation was observed between the plasma and spot urine ANP concentrations in simultaneously collected samples (unpublished report).

In summary, both plasma and urine ANP concentrations in patients with hyperthyroidism were increased in comparison with normal subjects. However, as the physiological significance of urine ANP is unknown, further studies are obviously needed.

References

- Amidi, M., D. F. Leon, W. J. DeGroot, F. W. Kroets and J. J. Leonard (1968). Effect of the thyroid state on myocardial contractility and ventricular ejection rate in man. *Circulation* 38, 229-239.
- Anderson, J. V., A. E. G. Raine, A. Proudler and S. R. Bloom (1986 a). Effect of haemodialysis on plasma concentrations of atrial natriuretic peptide in adult patients with chronic renal failure. *J. Endocrinol.* 110, 193-196.
- Anderson, J. V., J. Dockier, W. J. Mackenna and S. R. Bloom (1986 b). The plasma release of atrial natriuretic peptide in man. *Clin. Sci.* 71, 151-155.
- Argentin, S., J. Drouin and M. Nemer (1987). Thyroid hormone stimulated rat pro-natriodilatin mRNA levels in primary cardiocyte cultures. *Biochem. Biophys. Res. Commun.* 146, 1336-1341.
- Buccino, R. A., J. F. Spann, E. H. Sonnenblick and E. Braunwald (1967). Influence of the thyroid state in intrinsic contractile properties and energy of the myocardium. *J. Clin. Invest.* 46, 1669-1682.
- Burnett, Jr. J. C., P. C. Kao, D. C. Hu, D. W. Hesser, D. Heublein, J. P. Granger, T. J. Opge-north and G. S. Reeder (1986). Atrial natriuretic peptide elevation in congestive heart failure in the human. *Science* 231, 1145-1147.
- Cusson, J. R., J. Gutkowaka, E. Rey, N. Michon, M. Boucher and P. Larochelle (1985). Plasma concentrations of atrial natriuretic factor in normal pregnancy. *N. Engl. J. Med.* 313, 1230-1231.
- De Bold, A. J., A. T. Borenstein, A. T. Veress and H. Sonnenberg (1981). A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. *Life Sci.* 28, 89-94.
- Grossman, W., N. I. Robin, L. W. John, H. L. Brooks, H. A. Selenkow and L. Dexter (1971). The enhanced myocardial contractility of thyrotoxicosis. *Ann. Int. Med.* 74, 869-874.
- Kangawa, K. and H. Matsuo (1984). Purification and complete amino acid sequence of α human atrial natriuretic polypeptide (α hANP). *Biochem. Biophys. Res. Commun.* 118, 131-139.
- Kohno, M., T. Takaori, T. Matsuura, K. Murakawa, Y. Kanayama and T. Takeda (1986 a). Atrial natriuretic polypeptide in atrial and

- plasma in experimental hyperthyroidism and hypothyroidism. *Biochem. Biophys. Res. Commun.* 134, 178-183.
- Kohno, M., T. Matsuura, K. Yasunari, M. Yasuda, T. Takeda, Y. Nishizawa and H. Morii (1986 b). Increased circulating atrial natriuretic peptide in patients with thyrotoxicosis. *Arch. Intern. Med.* 146, 2077.
- Lang, R. E., H. Tholken, D. Graten, F. C. Luft, H. Ruskoaho and Th. Unger (1985). Atrial natriuretic factor—a circulating hormone stimulated by volume loading. *Nature* 314, 264-266.
- Marumo, F., H. Sakamoto, K. Ando, T. Ishigami and M. Kawakami (1986). A highly sensitive radioimmunoassay of atrial natriuretic peptide (ANP) in human plasma and urine. *Biochem. Biophys. Res. Commun.* 137, 231-236.
- Ohashi, M., N. Fujio, H. Nawata, K. Kato, H. Ibayashi, K. Kangawa and H. Matsuo (1987). High plasma concentrations of human atrial natriuretic polypeptide in aged men. *J. Clin. Endocrinol. Metab.* 64, 81-85.
- Rascher, W., T. Tulassay and R. E. Lang (1985). Atrial natriuretic peptide in plasma of volume-overloaded children with chronic renal failure. *Lancet* 11, 303-305.
- Sefidpar, M., A. Walser, F. Burkart and D. Burckhardt (1971). Cardiac output in thyroid disease. *Experientia* 28, 915-916.
- Suzuki, Y., R. Ohtake, H. Kobori, H. Suzuki and S-I. Shimoda (1987). A radioimmunoassay for α human atrial natriuretic peptide (α hANP) in plasma and urine. *Dokkyo J. Med. Sci.* 14, 127-133.
- Tang, J., R. J. Webber, D. Chang, J. K. Chang, J. Kiang and E. T. Wei (1984). Depressor and natriuretic activities of several atrial peptides. *Regul. Peptide.* 9, 53-60.
- Tikkanen, I., F. Fyhrquist, K. Metsarinne and R. Leidenius (1985). Plasma atrial natriuretic peptide in cardiac disease and during infusion in healthy volunteers. *Lancet* ii, 66-69, 1985.
- Zimmerman, R. S., H. Gharib, D. Zimmerman, D. Heublein and J. C. Burnett, Jr. (1987). Atrial natriuretic peptide in hypothyroidism. *J. Clin. Endocrinol. Metab.* 353-355.