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ATRIAL NATRIURETIC POLYPEPTIDE IN ATRIA AND PLASMA IN EXPERIMENTAL HYPERTHYROIDISM AND HYPOTHYROIDISM

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To investigate the involvement of thyroid hormone on the release of atrial natriuretic polypeptide (ANP), we have measured immunoreactive ANP in the atria and plasma of experimental hyperthyroid and hypothyroid rats. Plasma ANP was higher ($p<0.05$) in hyperthyroid rats and was lower ($p < 0.05$) in hypothyroid rats than in euthyroid rats. ANP content and concentration in the atria were lower ($p<0.01$) in hyperthyroid rats than in hypothyroid rats. An inverse correlation was found between the plasma ANP and ANP concentration in the atria ($n=15$, $r=-0.60$, $p<0.01$). The results indicate an increased systemic release of ANP from the atria in hyperthyroidism and a decreased systemic release in hypothyroidism.

Several peptides that have potent natriuretic and diuretic effects as well as vasodilatory action have been isolated from mammalian cardiac tissue (1-4). The intravenous infusion of synthetic atrial natriuretic polypeptide (ANP) in rats and humans causes a remarkable increase in urine flow and urinary excretion of sodium as well as a rapid decrease in arterial blood pressure (5,6). On the other hand, thyroid dysfunction is known to be associated with alterations in renal function, the cardiovascular system and total body water (7). Urine flow is reduced in hypothyroidism and increased slightly in hyperthyroidism (7). However, some is unknown of the mechanism in these alterations in relation to thyroid dysfunction. The present study examined the possible influence of the thyroid hormone on the release of ANP. We have measured ANP levels in the plasma and atria of experimental hyperthyroid and hypothyroid rats, using a sensitive radioimmunoassay.

Materials and Methods

Experimental animals

Fifteen male Wistar rats weighing between 190 and 210g were kept on a normal sodium diet and tap water ad libitum. Hyperthyroidism (n=5) was

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induced by daily intraperitoneal injection of L-thyroixine (T_4, from Sigma Chemical Co.) at a dose of 50ug/100g BW for twelve days . T_4 was dissolved in a weak basic saline solution (pH 8.5). Hypothyroidism (n=5) was induced by daily intraperitoneal injection of propylthiouracil (PTU, Sigma Chemical Co.) at a dose of 2mg/100g BW for twelve days. PTU was also dissolved in the saline solution as above. Control rats (n=5) received only the same saline solution intraperitoneally for twelve days . The daily dosage of injected saline solution was the same in all three groups. This was done because it has been reported that the ANP in the atria and plasma is directly related to the sodium balance in rats (8). At the end of all three treatments, the systolic blood pressure and heart rate were measured by the tail cuff method using a programmed sphygmomanometer (Narco Biosystems USA).

Preparation of samples

Blood samples for the determination of serum T_4 and plasma ANP were collected by rapid decapitation after hemodynamic study. Five ml of blood was drawn immediately into ice-chilled siliconized disposable glass tubes containing Trasylol (500 K.I.U/ml) and EDTA (1mg/ml). The plasma was separated by centrifugation for 10 minutes at 4°C, then immediately frozen and stored at -80°C for several days. Tissue was prepared by the modified method of Gutkowska et al (9). The hearts were removed and weighed immediately after the blood samples were taken. After washing them in a cold 0.1M sodium phosphate buffer, pH 7.4, the atria and ventricles were carefully dissected. Two atria from each rat were homogenized in 2 ml of 0.1M acetic acid for 60 sec and centrifuged for 20 min at 30,000 rpm. The supernatants were lyophilized, reconstituted in an assay buffer, and subjected to a radioimmunoassay to measure ANP.

Extraction of immunoreactive ANP (IR-ANP) from plasma

IR-ANP was extracted from rat plasma according to the modified method previously reported by Lang. et al.(10). From each plasma sample, 2 ml was diluted with 3 vol of 4% acetic acid. After centrifugation, the solution was pumped at a rate of 1ml/min, through a Sep-Pak C-18 cartridge (Waters Associates, Milford, MA) filled with octadecylsilane-C18(ODS-C18). After washing them with 5 ml of water, the absorbed peptides were eluted with 86% ethanol in 4% acetic acid. After the evaporation by a centrifugal evaporator (Model RD-31)(Yamato Scientific CO., Japan), the dry residue was dissolved in an assay buffer. The recovery rate was calculated by adding two different quantities of cold alpha rat ANP (1-28) (100 pg/ml and 200 pg/ml) to the plasma with dextran-coated charcoal. The recovery rate was 63.9 $\pm$ 3.6%.

Radioimmunoassay

The plasma IR-ANP was determined using antibodies against synthetic human ANP antibodies (Peninsula Laboratories) and ^{125}I-alpha human ANP (Peninsula Laboratories) as a tracer. This antibody cross-reacts 100% with alpha rat ANP (1-28) and (5-28), 57% with alpha rat ANP (18-28), 27% with alpha rat ANP (5-27) and 3% with alpha rat ANP (5-25). The antibody does not cross-react with somatostatin, oxytocin and vasopressin.The radioimmunoassay was performed in an assay buffer of 0.01M sodium phosphate, pH7.4, containing 0.05M NaCl, 0.1% BSA, 0.1% Non-diet NP-40 and 0.01% NaN . Rehydrated antiserum (100 µl) was added to 100 µl of the sample or to 100 ul of the standard ANP prepared in an assay buffer and then incubated for 24 hours at 4°C. Approximately 14,000 cpm ^{125}I-alpha human ANP was added to each reaction and incubated for an additional 24 hours. After the second 24-hour incubation, 100 µl of diluted normal rabbit serum and 100 µl of diluted goat anti-rabbit IgG serum were added and again incubated for 24 hours. After the third 24-hour incubation, the precipitate was collected by

centrifugation at 1700 Xg for 30 min. The supernatant was removed by aspiration and the pellet was counted for ^{125}I using a gamma counter. The effective range of the standard curve was between 5 and 200 pg of alpha human ANP per assay tube — 50% intercept, 22 pg of alpha human ANP. The inter-assay variation was 14.5% (N=10) and the intra-assay variation was 6.3% (N=18). Serum T was measured by radioimmunoassay.

Statistical analysis was done by an unpaired t-test or linear regression analysis.

Results

Our results are summarized in Table 1. The mean body weight in the hyperthyroid rats was significantly lower than in the euthyroid rats, whereas the mean body weight in the hypothyroid rats was the same as in euthyroid rats. The blood pressure and heart rate were higher in the hyperthyroid rats than in the euthyroid rats ($p < 0.001$), whereas no significant difference in blood pressure or heart rate was observed between hypothyroid and euthyroid rats. Plasma ANP was significantly higher ($p < 0.05$) in hyperthyroid rats and significantly lower ($p < 0.05$) in hypothyroid rats, than in euthyroid rats. ANP concentration in the atria was significantly lower in hyperthyroid rats than in hypothyroid rats ($p<0.01$). The ANP content in the atria was also lower in hyperthyroid rats than hypothyroid rats ($p<0.01$). As shown in Figure 1 and 2 respectively, significant inverse correlations were found between plasma ANP and ANP concentration in the atria ($n=15$, $r=-0.60$, $p<0.01$) and between plasma ANP and total ANP content in the atria ($n=15$, $r=-0.55$, $p<0.05$). Futhermore, a

Table 1: Effect of different thyroid states on body weight, hemodynamics, plasma ANP and ANP levels in atria

	Euthyroid (n=5)	Hyperthyroid (n=5)	Hypothyroid (n=5)
Serum T_4, ug/dl	5.6 ± 0.7	18.0 ± 1.8 [a]	1.8 ± 0.4 [a]
Weight, g	221 ± 15	180 ± 23 [b]	197 ± 16
BP, mmHg	127 ± 5	174 ± 5 [a]	128 ± 6
HR, beats/min	396 ± 22	540 ± 19 [a]	401 ± 22
Plasma ANP, pg/ml	156.3 ± 20.3	212.7 ± 36.2 [c]	102.6 ± 37.0 [c]
ANP in atria			
concentration, ug/g·tissue	162.9 ± 36.0	123.6 ± 52.9 [d]	261.8 ± 79.0 [e]
content, ug/rat	4.7 ± 1.5	3.2 ± 1.2 [d]	7.6 ± 3.1 [e]

Values are the mean ± S.D. Abbreviation are BP, Blood pressure; HR, heart rate a $p < 0.001$ vs euthyroid, b $p<0.05$ vs euthyroid, c $p < 0.05$ vs euthyroid, d $p < 0.01$ vs hypothyroid, e $p < 0.05$ vs euthyroid.

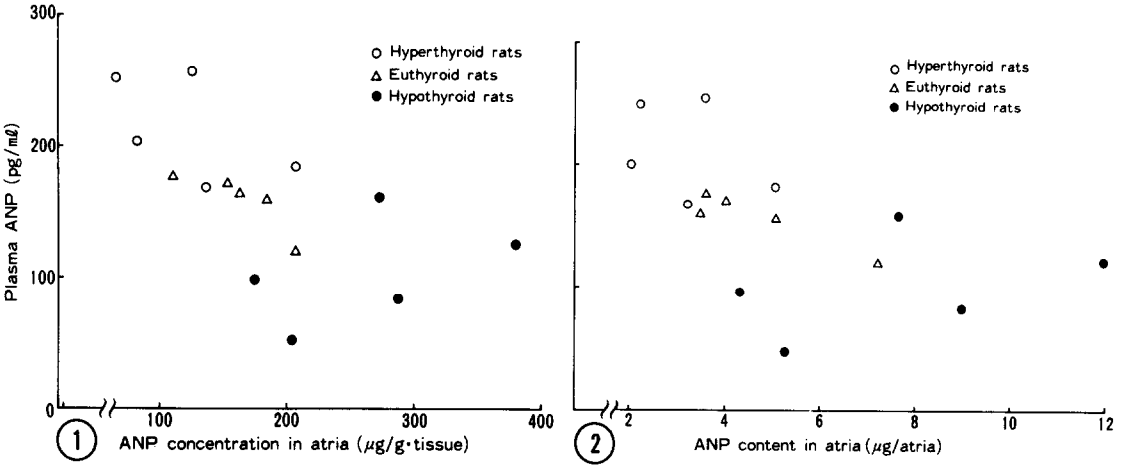

Figure 1: Correlation between plasma ANP and ANP concentration in atria in hyperthyroid, euthyroid and hypothyroid rats. A significant inverse correlation was found (n=15, r=-0.60, p<0.01).

Figure 2: Correlation between plasma ANP and ANP content in atria in hyperthyroid, euthyroid and hypothyroid rats. A significant inverse correlation was found (n=15, r=-0.55, p<0.05).

positive correlation was found between serum T_4 and plasma ANP (n=15, r=0.81, p<0.01)(Figure 3).

Discussion

The present study demonstrated that circulating ANP is elevated and ANP in the atria is decreased in hyperthyroidism, and that circulating ANP is decreased and ANP in the atria is increased in hypothyroidism. An inverse correlation between plasma ANP and ANP in the atria was observed. These

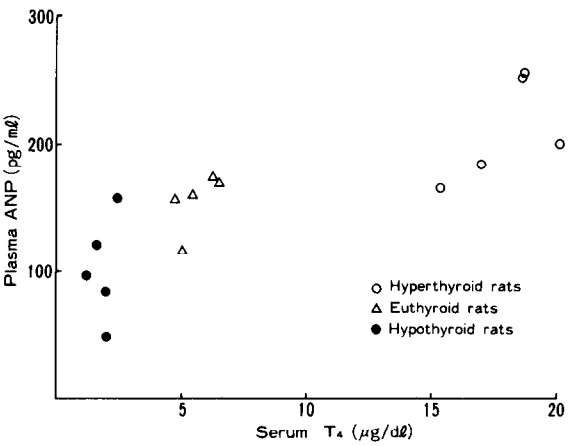

Figure 3: Correlation between serum T_4 and plasma ANP in hyperthyroid, euthyroid and hypothyroid rats. A positive correlation was found (n=15, r=0.81, p<0.01).

results indicate that ANP release is chronically stimulated in hyperthyroidism and is chronically suppressed in hypothyroidism. The observed alterations of systemic ANP release in different thyroid states may be explained by several mechanisms. One is the primary action of the thyroid hormone which accelerates the ANP release from the atria into the circulation. In fact, a direct positive correlation was observed between serum T_4 and plasma ANP in the present study.

Another possible mechanism is the influence of endocrine and adrenergic nervous systems, accompanied by thyroid dysfunction, on the systemic ANP release. Various endocrine abnormalities including impairment of the renin-angiotensin-aldosterone system have been reported in hyperthyroidism and hypothyroidism (7). Plasma renin activity is increased in hyperthyroidism, whereas it is decreased in hypothyroidism (7). Increased adrenergic activity is evident in the thyrotoxic state (7).

Secondary hemodynamic changes associated with thyroid dysfunction might also influence systemic ANP release providing a third possible mechanism. An elevated plasma ANP concentration was reported in a patient with paroxysmal atrial tachycardia (11) and in patients who had undergone atrial pacing (12). We also found that the plasma ANP concentration is significantly elevated in Spontaneously Hypertensive rats at 20 weeks of age (blood pressure 184 $\pm$ 7 mmHg) compared with age-matched Wistar-Kyoto rats (blood pressure 114 $\pm$ 5 mmHg)(unpublished observation). These data may suggest that tachycardia or high blood pressure is associated with increased systemic ANP release. In fact, tachycardia and an elevation of blood pressure were observed in hyperthyroid rats. However, we cannot determine whether the effects of thyroxine on the ANP release is primary or secondary. Furthermore, we cannot exclude the possibility that PTU treatment for a longer duration may decrease the ANP content in the atria, since the synthesis of protein and lipid are decreased in hypothyroidism. Although the precise mechanism of the effects of thyroid hormone on the ANP release remains to be clarified, our data indicate that systemic ANP release is

stimulated in hyperthyroidism and is suppressed in hypothyroidism, and that thyroxine is one of the regulatory factors in the release of ANP. We speculate that the changes in the ANP release in thyroid dysfunction is, at least in part, associated with alterations in the renal function and cardiovascular system that accompany this disease.

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References

1. Kanagawa, K and Matuo, H. (1984) Biochem Biophys Res Commun 118, 131-139.
2. Altas, SA, Kleinert, HD, Camargo, MJ, Januszesics, A., Sealey J.E., Laragh, J.H., Schilling, J.W., Lewicki J.A. Johnson, L.K and Mack,.T. (1984) Nature 309, 717-719
3. Currie, M.G., Geller, D.M., Cole BR, Siegel, N.R., Fok K.F., Adams, S.P., Eubanks, S.R., Galluppi, GR and Needleman. (1984) Science 223, 67-69.
4. Geller, D.M., Currie, M.G., Wakitani, K., Cole, B.R., Adams, S.P., Fok, K.F., Siegel, N.R., Eubanks, S.R., Galluppi, G.R. and Needleman, P.(1984) Biochem Biophys Res Commun 120, 333-338.
5. Currie, M., Geller, D.M., Cole B.R., Boylak, J.G., Yusheng, W., Holmberg, S.W. and Needlleman. (1983) 222, 71-73.
6. Richards, A.M., Nicholes, M.G., Ikram, H., Webster, M.W.I., Yandle, T.G. amd Espiner, E.A. (1985) Lancet 9, 545-548.
7. Ingbar, S.H. and Woeber K.A. (1974) Textbook of Endocrinology, pp157-162, pp191-197, W.B. SAUNDERS, Philadelphia.
8. Tanaka, I., Misono,K.S. and Inagami, T. (1984) Biochem Biophys Res Commun 124, 663-668.
9 Gutkowska, J., Thibault, G., Januszewics, P., Cantin, M. and Genest, J. (1984) Biochem Biophys Res Commun 122, 593-601.
10. Lang, R.E., Tholken H., Ganten, D., Luft, F.C., Ruskoako, H. and Unger, T.H. (1985) Nature, 314 264-266.
11. Schiffrin, E.L., Gutkowska, J., Kuchel, O., Cantin, M. and Genest, J. and Genest, J. (1985) New Engl J Med 312, 1196.
12. Gutkowska, J., Bourassa, M., Roy, O., Thibault, G., Garcia, R., Cantin, M. and Genest J. (1985) Biochem Biophys Res Commun 128, 1350-1357.