In order to clarify the effect of thyroid hormone on the plasma atrial natriuretic peptide (ANP) concentration, 14 patients with Graves' disease and 6 normal control subjects were studied. They were all under constant sodium intake because dietary sodium is known to affect the amount of plasma ANP. Sodium intake remained constant at 171 mEq daily for five consecutive days at which time the ANP concentration was measured. Graves' disease patients were tested both before and after surgery. The preoperative, hyperthyroid ANP level concentration in Graves' disease patients was $6.7 \pm 2.3$ fmol/ml compared to a significantly lower level of $4.2 \pm 1.4$ fmol/ml in normal control subjects. Seven days after surgery when Graves' disease patients became euthyroid their ANP markedly decreased to $4.2 \pm 2.9$ fmol/ml.

In the present study we were able to confirm that under a constant sodium diet, high plasma ANP in patients with Graves' disease was reduced after surgery when they became euthyroid. Results also suggest that high circulating ANP might play an important role in sodium and water metabolism and hemodynamic changes in hyperthyroidism.
Landenson et al., 1986) and hyperthyroid man (Kohno et al., 1987; Woolf and Moult, 1987). Opposite results in man were then reported by Landenson et al. (1987). We conjectured that these discrepancies were due to two main factors: the amount of dietary sodium intake and the method of measuring ANP.

Sagnella et al. (1987) reported that the plasma ANP concentration rises with increased dietary sodium intake. And we observed first hand the direct correlation between dietary sodium intake and the urinary sodium concentration which ranged from 30 to 500 mEq/g creatinine in outpatients to our clinic. We therefore felt the necessity to regulate dietary sodium in patients with Graves’ disease where the effect of thyroid hormone plays heavily on the ANP concentration. We also developed a sensitive RIA measurement with monoclonal antibody to alpha ANP (Naomi et al., 1987; Naomi et al., 1988). In this manner we were able to study the effect of thyroid hormone, on the plasma ANP concentration measured by this new sensitive method, in Graves’ disease when dietary sodium intake was constant.

**Patients and Methods**

After receiving informed consent, 14 patient with Graves’ hyperthyroidism (4 males and 10 females, aged 34.1±12.3 years) were admitted to Noguchi Thyroid Clinic and Hospital Foundation for surgical treatment and study. No patient had taken any antithyroid drugs before admission. After admission, methyl-mercaptoimidazole (MMI) 30 mg daily was administered for about one month until the time of surgery. A pre-operative study was carried out within one week of admission. All patients were in a hyperthyroid state in spite of pre-operative MMI therapy. This was most likely due to the short duration of drug administration. Grave’s disease was the primary diagnosis in all. None showed signs of heart failure, valvular heart disease or atrial fibrillation. The diagnosis was based on serum free triiodothyronine (FT3), free thyroxine (FT4), thyrotropin (TSH) and radioactive iodine uptake (RAIU). All had normal plasma urea and electrolytes values. Patients receiving diuretics or antihypertensive drugs were not included in this study.

In order to examine the effect of dietary sodium intake on plasma ANP, 6 normal control subjects (2 males and 4 females age 30.0±5.2), were tested over a 15-day period with low (34.2 mEq/day), moderate (171 mEq/day) and high (342 mEq/day) intakes of sodium. The ANP concentration was measured on the fifth day of each diet. Graves’ disease patients received moderate sodium intake only. Their diet remained constant at 171 mEq/day for five days before surgery and was resumed at 3 days after surgery for another five days. Plasma ANP, serum free thyroxine (FT4), free triiodothyronine (FT3) and thyrotropin (TSH) were measured in both the pre-surgery, hyperthyroid state and the post-surgery euthyroid state. On the last day of the pre and post surgery sodium constant diets, 24 hours urine collectin was made and measured for sodium, potassium and creatinine excretion. Blood pressure and heart rate were measured and peripheral venous blood samples were drawn in the supine position for more than five minutes. All examinations were carried out in the early morning.

Blood samples to determine immunoreactive (IR)-ANP were taken into prechilled tubes containing disodium EDTA (1 mg/ml) and aprotinin (500 KIU/ml). Samples were immediately centrifugated at 4°C, and the plasma was separated and frozen at −20°C until extraction. ANP was measured according to the method already published by Naomi et al. (1988). Serum FT3 and FT4 were measured with commercially available RIA kits (Amerlex FT4 and FT3, from the Radiochemical Center, Amersham International, Amersham, England). Serum TSH was determined by immunoradiometric assay (IRMA) (Dainabot Radioisotope Laboratories, Tokyo). Other biochemical measurements were done with an automatic analyser.

Paired and Student’s t-test and Chi square test were used for statistical analysis. Each value was expressed as the mean±SD.
Results

The Plasma ANP concentration in normal control subjects significantly rose in accordance with increasing dietary sodium intake; with levels of $3.4 \pm 0.9$ fmol/ml on the fifth day of the low sodium diet (34.2 mEq/day), $4.2 \pm 1.4$ fmol on the fifth day of the moderate sodium diet (171 mEq/day) and up to $8.3 \pm 3.5$ fmol/ml on the fifth day of the high sodium diet (342 mEq/day).

There was a significant correlation between plasma ANP and serum FT4 ($r = 0.52$, $p < 0.02$) and FT3 ($r = 0.47$, $p < 0.05$). However, no significant relationship was found between plasma ANP and systolic blood pressure, diastolic pressure and, pulse rate, cardio-thoracic ratio (CTR) and age.

Dietary Na intake (171mEq/day) for 5 days.

![Graph showing plasma ANP concentration before and one week after surgery under a constant dietary sodium intake (171 mEq/day) in 14 patients with Graves' disease. Note that the preoperative ANP concentration was significantly reduced after surgery when patients become euthyroid.]

Fig. 1. Plasma ANP concentration before and one week after surgery under a constant dietary sodium intake (171 mEq/day) in 14 patients with Graves' disease. Note that the preoperative ANP concentration was significantly reduced after surgery when patients become euthyroid.
Table 1. Clinical and laboratory profiles before and after surgery in 14 patients with Grave's disease under a constant dietary sodium intake (171 mEq/day for 5 days)

<table>
<thead>
<tr>
<th></th>
<th>Before surgery (hyperthyroid)</th>
<th>After surgery (euthyroid)</th>
<th>Normal range</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP (fmol/ml)</td>
<td>6.7±2.3</td>
<td>4.2±2.9</td>
<td>2.3–5.4</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>142.3±17.2</td>
<td>126.5±17.8</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>88.5±10.5</td>
<td>78.5±8.0</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Sodium output (mEq/day)</td>
<td>137.4±36.3</td>
<td>109.9±43.7</td>
<td>p&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Creatinine output (mg/day)</td>
<td>894.8±291.9</td>
<td>918.4±411.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Urine volume (ml/day)</td>
<td>1133.3±437.5</td>
<td>1132.3±451.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Free T3 (pg/ml)</td>
<td>11.6±2.8</td>
<td>2.8±1.3</td>
<td>2.7–5.9</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Free T4 (ng/dl)</td>
<td>4.4±2.1</td>
<td>1.2±0.7</td>
<td>0.7–1.8</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>TSH (µU/ml)</td>
<td>0.07±0.13</td>
<td>0.05±0.08</td>
<td>0.3–3.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

T3; triiodothyronine  T4; thyroxine  TSH; thyrotropin
Each value is expressed as the mean±SD.

As shown in Figure 1, plasma ANP significantly decreased after surgery. The preoperative plasma ANP concentration in 14 Graves' disease patients was higher than in the 6 normal controls when both groups were on a constant diet of 171 mEq/day sodium for five days. Table 1 shows clinical and laboratory profiles before and after surgery in the 14 Graves' disease patients. Pre-operative plasma ANP, systolic blood pressure, heart rate, sodium output, FT3 and FT4 significantly decreased after surgery, while creatinine output, urine volume and TSH were unchanged.

Discussion

The administration of thyroid hormone increased the amount of plasma ANP in rats (Kohno et al., 1986). Matsubara et al. (1987) reported that thyroid hormone directly stimulates the synthesis of ANP by rat atrial myocytes. Moreover, Gardner et al. (1987) demonstrated that T3 administration induced both increased ANP release and increased m-RNA in an in vitro neonatal cardiomyocyte system. This explains how increased plasma ANP in hyperthyroid rats was attributed to the direct effect of thyroid hormone on cardiomyocytes.

The relationship between plasma ANP and thyroid hormone in man has been controversial. Kohno et al. (1987) reported increased plasma ANP in patients with hyperthyroidism which correlates with serum free T4 and free T3, and decreased ANP after antithyroid drug treatment. However, Landenson et al. (1987) reported that hyperthyroidism and hypothyroidism in man were not associated with a significantly altered plasma ANP concentration. These discrepancies might be due to the method of ANP measurement and the absence of constant of dietary sodium intake. In the present study, we eliminated the two above-mentioned points. Firstly, we developed a high sensitive RIA method for measuring ANP with monoclonal antibody to alpha-hANP, and measured plasma ANP by this method. Secondly, we measured plasma ANP on a constant sodium diet of 171 mEq/day.

In a preliminary unpublished study conducted at our clinic we measured plasma ANP in 21 out-patients with Graves' disease and 17 normal control subjects without enforcing any regulations for constant dietary sodium intake. The plasma ANP concentration in 21 untreated Graves' disease patients (4.1±1.6 fmol/ml) was not significantly different from that in 17 normal
plasma ANP and conversely low sodium intake lowered plasma ANP in normal subjects (Sagnella et al., 1987). Our data confirmed that report. Therefore, we began to act on the assumption that sodium intake might affect the amount of ANP in patients with Gravé's disease. The effect of dietary sodium on plasma ANP might overwhelm the effect of the thyroid hormone. When Gravé’s patients’ dietary sodium intake was constant, the plasma ANP concentration which was significantly higher than that of normal subjects on the same dietary sodium intake, decreased after surgery. Therefore, we concluded that sodium intake was an important factor which influenced these results.

We found that on a constant sodium diet, high plasma ANP in patients with Gravé’s thyrotoxicosis decreased after surgery when patients became euthyroid. We conclude from these observations that a high circulating concentration of ANP might play an important role in sodium and water metabolism and hemodynamic changes in hyperthyroidism.

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