**In Vitro Study on Release of Thyroid Hormone in Solitary Autonomously Functioning Thyroid Nodules Using Cell Culture Method**

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**Abstract**

In vitro release of thyroid hormone was investigated under basal and TSH-stimulated conditions in the solitary autonomously functioning thyroid nodules (AFTN). A small portion (0.5 g of wet weight) of the nodules and adjacent thyroid tissues removed surgically from five patients with solitary AFTN were prepared for the dispersed cell culture. In the experiment on non TSH-stimulated (basal) conditions, those culture media which were totally replaced on the 5th day after primary culture were utilized for the determination of thyroxine (T4) and triiodothyronine (T3) by radioimmunoassay. T4 and T3 levels in culture media of the functioning nodules were 1.15±0.33 μg/dl (mean ±SEM) and 2.72±0.68 ng/ml, contrasted with levels of 0.67±0.09 μg/dl and 1.24±0.22 ng/ml in the paranodular tissues. The mean ratios of T3/T4 of the nodules and paranodular tissues were 0.25±0.02 and 0.19±0.02, respectively (p<0.05). Meanwhile, in another experiment under TSH stimulatory conditions employing 40 and 80 μU/ml of human TSH, there were no significant differences in T4 and T3 releases when the two groups were compared.

Although endocrinologic and pathologic observations of the autonomously functioning thyroid nodules (AFTN) have been increasingly refined, biochemical studies using in vitro systems are extremely few. Some investigators employed iodine organification, thyrotropin (TSH)-induced adenylate cyclase activity, glucose oxidation and thyroglobulin biosynthesis in the thyroid tissues as biochemical parameters for the estimation of synthesis of thyroid hormone in the functioning nodules (Burk and Szabo, 1972; Larsen et al., 1973; Monaco et al., 1975). The present investigation, therefore, was attempted to make clear the secretory modes of thyroid hormone in the solitary AFTN, using the procedures of direct measurement of thyroxine (T4) and triiodothyronine (T3) released into the culture media under basal and TSH-stimulated conditions. Furthermore, clinical observation of the syndrome of T3 thyrotoxicosis was also carried out on patients with AFTN.

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Materials and Methods

Subjects
The patients reported on here include three with non-toxic solitary AFTN and two with toxic type. They consisted of four females and one male. Their ages ranged from 30 to 73 years (Table 1). The diagnosis of solitary AFTN was made according to the accepted clinical and laboratory criterias. All of the patients underwent surgery without any preoperative treatment or preparations.

In vitro studies
Small pieces of tissue (approximately 0.5 g of wet weight) taken from the hyperfunctioning nodule and paranodular normal thyroid gland at surgery, were placed immediately in cold (4 C) Eagle's MEM supplemented with 10% decplemented fetal calf serum containing 100 units/ml of Penicillin G and 100 µg/ml of Streptomycin. After trimming of connective tissues, the specimens were then minced into 0.5 to 1.0 mm³ fragments with a surgical scalpel. Dispersed thyroid cells were prepared by digestion with 0.05% trypsin (Difco Lab., U.S.A.) and washed twice with the same medium. Thereafter, they were suspended at a concentration of 1×10⁶ cells per ml in culture medium. Cell culture was subsequently carried out at 37 C under the closed system. Culture media were totally replaced every 5 days.

In the experiment under the basal (non-stimulated) conditions, the 5th day's culture media after primary culture were employed for the determination of T4 and T3. On the other hand, in the another experiment under TSH stimulatory conditions, two doses of human TSH (Daiichi, Japan) 40 and 80 µU/ml, were added to the new culture media for one hour cell-stimulation study. In addition, before each stimulation study, another one hour cell culture with renewed culture medium alone was performed because of setting up control. The results were expressed as a percentage of the "basal" level of the medium alone control culture (% increase).

The concentrations of T4 and T3 in the culture media were measured by radioimmunoassay using SPAC T4 (Daiichi, Japan) and T-3 (Dainabot, Japan) RIA Kits, respectively. For statistical analysis, Student's t-test was used. A P value less than 0.05 was considered statistically significant.

Results

Laboratory data for the patients with solitary AFTN
Table 1 shows clinical, biochemical and histological findings for the five patients included in the present study. In two out of 5 patients (No. 1 and 2), mild thyrotoxic

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Serum T₄ (µg/dl)*</th>
<th>Serum T₃ (ng/ml)**</th>
<th>Serum TSH (µU/ml)***</th>
<th>Histologic Diagnosis</th>
</tr>
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<tr>
<td>1</td>
<td>F</td>
<td>42</td>
<td>12.1</td>
<td>2.1</td>
<td>3.5</td>
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</tr>
<tr>
<td>2</td>
<td>M</td>
<td>46</td>
<td>11.4</td>
<td>4.4</td>
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</tr>
<tr>
<td>3</td>
<td>F</td>
<td>30</td>
<td>7.6</td>
<td>1.6</td>
<td>2.5</td>
<td>Papillary adenoma</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>73</td>
<td>7.5</td>
<td>1.3</td>
<td>4.0</td>
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</tr>
<tr>
<td>5</td>
<td>F</td>
<td>48</td>
<td>7.7</td>
<td>1.1</td>
<td>5.0</td>
<td>Colloid adenoma</td>
</tr>
</tbody>
</table>

* Normal range for serum T₄ is 5.1-12.8 µg/dl.
** Normal range for serum T₃ is 0.96-1.92 ng/ml.
*** Normal range for serum TSH is less than 10 µU/ml.
Symptoms were observed with normal T4 and elevated T3 values, respectively. The remainder was clinically and biochemically non-toxic. Histologically, 4 were colloid adenomas and one a papillary adenoma.

Levels of T4 and T3 in culture media in basal condition

As shown in Fig. 1, the mean values for T4 and T3 in the culture media of the five functioning nodules were 1.15±0.33 µg/dl (mean±SEM) and 2.72±0.68 ng/ml, respectively. Those for the paranodular thyroid tissues were 0.67±0.09 µg/dl and 1.24±0.22 ng/ml, respectively. Statistically, no significant differences were observed in T4 and T3 releases between the autonomously functioning nodules and the paranodular thyroid tissues.

T3/T4 secretion ratio

The mean values for T3/T4 were 0.25±0.02 in the functioning nodule and 0.19±0.02 in the paranodular thyroid tissue (Fig. 2). Statistical difference was observed in the mean values between both groups (p<0.05).

Increases of T4 and T3 releases by TSH stimulation

TSH-induced releases of T4 and T3 were represented by % increase, as shown in Fig. 3. The non-pharmacological doses of human TSH demonstrated stimulatory effects in synthesizing T4 and T3 by the cultured cells of the functioning nodules and paranodular thyroid tissues, respectively. However, those % increase values for both groups are not significantly different in T4 and T3 releases, respectively.
Discussion

The majority of studies on the synthesis of thyroid hormone in the AFTN have been carried on mainly from the viewpoints of iodine metabolism and thyroglobulin biosynthesis. In earlier reports on the in vitro responsiveness of AFTN to TSH stimulation, Larsen et al. (1973) and Burk and Szabo (1972) have used substantial amounts of TSH, i.e., non-physiological doses. Their observations demonstrated that the functioning nodules responded much more strongly than the surrounding tissues. Hence, they concluded that hyperresponsivity to TSH might play a role in the genesis of AFTN.

In the present study, both T4 and T3 were employed as biochemical indices of hormone production by the dispersed cells. From our observations on the release of T4 and T3 produced by the primarily cultured thyroid tissues, it would appear that both the nodular and paranodular cells were able to synthesize thyroid hormone as well. It is, however, of great interest the T3/T4 secretion ratio in the culture media of the functioning nodule was significantly higher than that of normal paranodular tissue. At present, it is generally believed that T3 thyrotoxicosis or hypertriiodothyroninemia in those patients with solitary AFTN will be seen clinically in a higher incidence (Hamburger, 1980). The foregoing results therefore suggest that the solitary AFTN might be associated with increased T3 production.

Meanwhile, there existed no significant increases in T4 and T3 release from the cultured cells of hyperfunctioning nodule compared with those of the paranodular tissues under the TSH-stimulatory condition. This finding may well be compatible with the clinical fact that a thyroid scintiscan after TSH administration demonstrates an altered distribution of the same isotope of iodine used for the original scan (Miller, 1978). It is difficult to give a satisfactory explanation for the discrepancies between our observation and those of two groups previously described. The following reasons may be offered: the differences in the dose of TSH added, culture systems employed and biochemical parameters determined.

Our studies indicate that the rate of T3 synthesis and its release from solitary AFTN appears to be greater than in the paranodular thyroid tissues. Moreover, hyperfunction of the nodules seems to be dependent on the autonomous mechanisms rather than...
hyperresponsiveness to the endogenous TSH. It is evident that further basic investigations are required to elucidate the precise mechanism of the genesis of AFTN.

References


