Increased Peroxidase Activity in Pendred’s Syndrome with Hypothyroidism

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Tohoku J. exp. Med., 1976, 119 (2), 103-113 — An 8-year-old boy with goiter and bilateral nerve deafness had a 46% discharge of radioiodine after thiocyanate administration. He was clinically euthyroid. Although the serum total $T_4$ was low (2.4 μg/100 ml) and TSH was significantly high (181 μU/ml), the serum total $T_3$ was normal (152 ng/100 ml). It was considered that the increased release of TSH by the feedback mechanism in response to the low $T_4$ resulted in a quite normal level of serum $T_3$. The thyroid gland demonstrated a low stable iodine content, an increase in MIT/DIT ratio and a decrease in iodothyronine. The thyroglobulin behaved normally in Sephadex G-200 chromatography and immunoreaction. Thyroid tissue exhibited increased peroxidase activity as measured by $I_3^-$ formation. Increased peroxidase activity may be related to the observed increase in serum level of TSH. — Pendred’s syndrome; thyroidal peroxidase activity

A defective organic binding of iodide has been found in goitrous patients with nerve deafness (Morgans and Trotter 1958). This disorder is known as Pendred’s syndrome. In spite of the defect of iodide binding, the concentration of serum PBI or $T_4$ remains normal in most patients (Stanbury 1972), although the low level of PBI in Pendred’s syndrome was reported by Johnsen (1957) and low serum level of $T_4$ and high level of TSH in Pendred’s syndrome with clinical euthyroidism were reported by Purves (1972).

Recently iodide peroxidase of the thyroid gland from patients with iodide organification defect has been studied (Haddad and Sidbury 1959; Ljunggren and Vecchio 1969; Hagen et al. 1971; Burrow et al. 1973; Ljunggren et al. 1973; Niepomnisczoe et al. 1972, 1973; Valenta et al. 1973). The thyroid tissue from the patients with Pendred’s syndrome who were clinically euthyroid with normal serum $T_4$ and $T_3$ resin uptake showed normal peroxidase activity (Burrow et al. 1973). But the study of peroxidase activity in Pendred’s syndrome with clinical euthyroidism and a high level of TSH has not been reported. This paper describes peroxidase activity in the thyroid gland of our patient with Pendred’s syndrome, who had a low level of $T_4$, normal $T_3$ and a high level of TSH.

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METHODS AND MATERIALS

Case report

An 8-year-old boy was admitted to our clinic in October, 1972. He was not brought up in an endemic goiter area. Moderate hearing loss since infancy and thyroid swelling only 3 months prior to his admission had been noticed. He was a product of normal full term pregnancy and delivery. His parents were first cousins. Among three siblings the eldest sister had moderate deafness but no goiter.

His intelligence and growth were normal. His height was 118.5 cm and weight was 23.3 kg. The thyroid was palpated diffuse, soft and enlarged, but neither thrill nor bruit was present. Though right cryptorchidism was found, the remainder of the physical examination was within normal limits. There was no clinical suggestion of hypothyroidism.

Laboratory examination

The routine laboratory studies were normal. His IQ was 90. The hearing loss was symmetrical and perceptive, greater for high tones than for low ones. Roentgenography of the hand revealed the bone age was 7.5 years old. He had normal 46 XY chromosomes.

In vivo studies

Serum T4 was measured by Tetrasorb kit (Dinabot Co.). Serum T3 was determined by radioimmunoassay (Sakurada et al. 1973). 131I-T3 resin uptake was assessed by Triosorb kit (Dinabot Co.). Serum TSH was measured by radioimmunoassay with HTSH kit (Daichi RI Lab.). Thyroxine-binding-globulin (TBG) was assessed by reverse flow paper electrophoresis (Tanaka and Starr 1959) and thyroxine-binding-prealbumin (TBPA) was assessed by polyacrylamide gel electrophoresis (Sakurada et al. 1967). After an oral administration of 131I, serial measurements of radioactivity were made over the thyroid. The thiocyanate discharge test was performed 6 hr after 131I administration. 1 g of potassium thiocyanate was administered orally and thyroidal counts were assessed at 30 min, 1, 1.5 and 2 hr. The effect of administered TSH on both uptake of radioiodine by the thyroid and release of thyroid hormone from the gland was studied. 131I 24 hr uptake was measured before and after daily administration of 7 USP units of TSH for 3 successive days. The serum T3 and T4 were estimated before, and at 3 and 11 hr after every administration of TSH.

Serum TSH levels were measured at 10, 20, 40, 60 and 90 min after intravenous administration of 500 µg of TRH (Tanabe Co.).

Upon the completion of the investigations, l-T3 was administered for about 2 months, and then replaced by l-T4. Serial measurements of 131I 24 hr uptake, T3, T4 and TSH were made during the treatments.

In vitro studies

Tissue source. A part of the thyroid gland of the patient was obtained surgically. A part of the specimen was examined histologically, and the remaining portion was promptly frozen on dry ice for further studies. Control studies were made on specimens of normal thyroid tissue from euthyroid subjects with nodular goiter, and on diffuse toxic goiter.

Analysis of thyroid digests. 48 hr before surgery, he was given an oral tracer dose of 131I. 500 mg of thyroid specimen was homogenized in 0.05 M phosphate buffer, pH 7.4, containing 0.01 M MgSO4 and was heated for 3 min at 100°C. Pronase (1 mg) was added to the homogenate. After 24 hr of incubation, the second addition of pronase (1 mg) was performed, and the homogenate was again incubated for additional 24 hr. The digest was chromatographed in n-butanol-acetic acid-water (78:5:17) using ascending chromatography on Whatman MM3 paper. The radioactivity was counted with a paper chromatogram scanner (Aloka Co.). The percentage of total counts as determined for each radioactive area. Iodine concentration per gram of wet thyroid tissue was determined by the method.
of Barker et al. (1951).

**Thyroid peroxidase preparation.** The thyroid peroxidase was prepared according to the method described by Niepomniszcz et al. (1969). Each tissue specimen was homogenized at 4°C with 4 ml of 0.15 M KCl solution with the use of a Potter-Elvehjem homogenizer with glass pestle. The homogenate was centrifuged at 600 × g for 10 min. The precipitate was discarded and the supernatant fluid was centrifuged at 15,000 × g for 30 min. The sediment was collected and referred to as mitochondria, although it probably contained also heavy microsomes and lysosomes. The mitochondria was washed twice with the KCl solution. 1 ml of 1%, digitonin in 0.05 M phosphate buffer, pH 7.0, was added to the mitochondria obtained from about 1 g of wet tissue and suspension was mixed with aid of a Potter-Elvehjem homogenizer. After 30 min incubation at 4°C, the suspension was centrifuged at 40,000 × g for 20 min and the supernatant solution was collected. Protein determination was carried out by the method of Lowry et al. (1951).

**Assay of peroxidase.** Iodide peroxidase was measured by a modification of the triiodide method of Alexander (1962). The peroxidase assay mixture was made in a 3-ml cuvette and contained 0.5 ml of 0.08 M KI, 2 ml of 0.05 M phosphate buffer, pH 7.0, and 50 μl of the solubilized enzyme. The absorbancy of this solution was set at zero (287.5 nm) and the reaction was started with the addition of 20 μl of 0.08 M H₂O₂. The increase in absorbance was recorded every 15 sec. Activity was expressed as the unit described by Niepomnischcz et al. (1969).

**Thyroglobulin studies.** The supernatant obtained at 15,000 × g centrifugation was centrifuged at 105,000 × g for 120 min. Thyroglobulin was isolated by gel filtration as follows: 0.5 ml of the 105,000 × g supernatant was applied to a Sephadex G-200 column (1-1/2 × 30 cm) and eluted with 0.1 M Tris-hydrochloric acid buffer, pH 7.4 (Lissitzky et al. 1968). Successive fractions of eluate were collected for protein determination. The eluted thyroglobulin was incubated for 1 hr at 37°C and 24 hr at 4°C with antisera from a patient with Hashimoto's thyroiditis to neutralize the thyroglobulin antibodies. The degree of neutralization was measured by inhibition of the tanned red cell thyroglobulin hemagglutination assay by Thyroid test kit (Fujizoki Co.).

**RESULTS**

**In vivo studies**

The laboratory data on thyroid function are presented in Table 1. The serum T₄ and ¹³¹I-T₁₃ resin uptake were low. The thyroidal radioactive iodine uptake was

<table>
<thead>
<tr>
<th>Table 1. Thyroid function tests of the patient with Pendred's syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMR</strong></td>
</tr>
<tr>
<td>¹³¹I-T₂ resin uptake</td>
</tr>
<tr>
<td>Serum total T₂</td>
</tr>
<tr>
<td>Serum total T₁</td>
</tr>
<tr>
<td>Serum TSH</td>
</tr>
<tr>
<td>10 min after TRH</td>
</tr>
<tr>
<td>20 min</td>
</tr>
<tr>
<td>40 min</td>
</tr>
<tr>
<td>60 min</td>
</tr>
<tr>
<td>90 min</td>
</tr>
<tr>
<td>24 hr thyroidal radioiodine uptake after TSH administration</td>
</tr>
<tr>
<td>TBG binding capacity</td>
</tr>
<tr>
<td>TBPA binding capacity</td>
</tr>
<tr>
<td>Antithyroglobulin antibody</td>
</tr>
</tbody>
</table>

Normal limits are indicated in the parentheses.
Fig. 1. Thyroidal uptake of orally administered $^{131}$I (●—●) and effect of oral administration of thiocyanate (○—○).

The gland's response to TSH was also evaluated from the changes in serum T$_3$ and T$_4$ (Fig. 2). In the control, T$_4$ increased significantly during the administration of TSH. The level of T$_3$ gradually increased with the peak or the maximum at 3 hr after every injection of TSH. In our patient the serum level of T$_4$ was not changed by TSH injection. The first injection of TSH produced a rapid increase of T$_3$ but a further increase was not generated by the 2nd and 3rd injections. The basal level of TSH was significantly high and a distinct release of TSH occurred immediately after TRH injection and the high level maintained for 90 min (Table 1).

The administration of T$_3$, 25 μg per day for 12 days and 50 μg per day for 10 days, lowered the serum TSH from 181 μU/ml to an undetectable level and $^{131}$I 24 hr uptake from 64% to 22% with a reduction of the size of the struma (Fig. 3). It was suggested that by the feedback mechanism involving T$_3$ and TSH secretion the...
Increased Peroxidase Activity in Pendred's Syndrome

Fig. 2. Changes in serum T₃ and T₄ after intramuscular injection of TSH, in normal control subject (---) and our patient (—).

Fig. 3. 24 hr thyroidal radioiodine uptake, serum T₃, serum T₄ and TSH during treatments in our patient with Pendred's syndrome.

TSH was increased and this increase caused the enlargement of the thyroid gland.

The thyroid function of the family was shown in Table 2. The eldest sister with moderate deafness had normal thyroid function tests. The thiocyanate discharge test was negative. The parents had also normal thyroid function.
TABLE 2. Thyroid function tests in a family with the Pendred’s syndrome

<table>
<thead>
<tr>
<th></th>
<th>BMR</th>
<th>24 hr thyroidal radiiodine uptake</th>
<th>T₄</th>
<th>KSCN test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>-8.5%</td>
<td></td>
<td>8.0 µg/100 ml</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>-4</td>
<td>18%</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Sister with deafness</td>
<td>-5</td>
<td>11.9</td>
<td>8.6</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Fig. 4. Microscopic appearance of the thyroid.

In vitro studies

Pathological examination. The tissue consisted of compactly arranged irregular-sized follicles. Large follicles were particularly striking. The follicle lining cells were cuboidal and some were imbedded into their luminal spaces. The follicles contained light-stained colloid. There was no evidence of chronic lymphocytic thyroiditis. The pathological diagnosis was struma colloides macrofollicularis (Fig. 4).

Analysis of thyroid digests. The distribution in the tissue of ¹³¹I given 48 hr before the biopsy was noted in Table 3. The MIT/DIT ratio was above 2.0 and only 2% of the radioactivity was in the form of iodothyronine. Total iodine concentration per gram of wet thyroid tissue was low (60.6 µg/g).

Peroxidase activity. Peroxidase activity was determined in the thyroid preparation from the patient with Pendred’s syndrome and compared with the activity in the thyroid tissue from patients with nontoxic nodular goiter and diffuse toxic goiter. The results from the determination of peroxidase are presented in Table 4. Normal thyroid tissue was obtained from the patient with nontoxic nodular goiter without administration of thyroid hormone. Peroxidase activity in the patient with Pendred’s syndrome was 6,311 units which was higher than the values in the control patients. The results indicated that the peroxidase content of the thyroid tissue with Pendred’s syndrome increased as compared with
Table 3. Percent distribution of $^{131}$I-labelled iodothyrosines and iodothyronines in pronase digested thyroid homogenate

<table>
<thead>
<tr>
<th></th>
<th>Iodide</th>
<th>MIT</th>
<th>DIT</th>
<th>$T_1+T_4$</th>
<th>MIT/DIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pendred's syndrome</td>
<td>9</td>
<td>66</td>
<td>23</td>
<td>2</td>
<td>2.87</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>3</td>
<td>42</td>
<td>45</td>
<td>10</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Solvent: n-butanol-acetic acid-water (78:5:17)

Table 4. Iodide peroxidase activity of human thyroid digitonin solubilized preparations

<table>
<thead>
<tr>
<th></th>
<th>Protein concentration (mg/ml)</th>
<th>Triiodide assay $\frac{\Delta OD}{15 \text{ sec}} \times 10$</th>
<th>Iodide peroxidase activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pendred’s syndrome</td>
<td>0.225</td>
<td>45</td>
<td>6.311</td>
</tr>
<tr>
<td>Normal tissue from nodular thyroid</td>
<td>1.07</td>
<td>50</td>
<td>1.558</td>
</tr>
<tr>
<td>Nodular tissue</td>
<td>1.00</td>
<td>50</td>
<td>1.440</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>1.50</td>
<td>120</td>
<td>2.133</td>
</tr>
</tbody>
</table>

The patient with hyperthyroidism was treated with antithyroid drug before surgery.

Fig. 5. Sephadex G-200 gel filtration of the $105,000 \times g$ supernatant.

not only normal tissue but also tissue from toxic goiter.

Thyroglobulin studies. Thyroglobulin was eluted from Sephadex G-200 in the same position as bovine thyroglobulin (Fig. 5). The eluted thyroglobulin, containing 1.4 mg of protein per ml, was incubated with antiserum. Increasing concentrations of thyroglobulin progressively neutralized antithyroglobulin antibodies in the Thyroid test (Table 5).
TABLE 5. Inhibition of thyroglobulin hemagglutination test by the thyroglobulin preparation of Pendred's syndrome

<table>
<thead>
<tr>
<th>Incubation mixture No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroglobulin preparation (μl)</td>
<td>80</td>
<td>50</td>
<td>20</td>
<td>—</td>
</tr>
<tr>
<td>Antiserum (μl)</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Buffer solution* (μl)</td>
<td>—</td>
<td>30</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>Thyroid test titer</td>
<td>1:1.600</td>
<td>1:6.400</td>
<td>1:25.600</td>
<td>1:102.400</td>
</tr>
</tbody>
</table>

The thyroglobulin hemagglutination titer was the minimal dilution of the mixture that produced a positive reaction according to the specifications of the Fujizoki Thyroid test kit. * 0.1 M Tris-hydrochloric acid buffer.

DISCUSSION

The patient with goiter and nerve deafness described here was clinically euthyroid, despite the low serum T₄. TSH concentration of the serum was significantly high and greatly increased in response to intravenous injection of TRH. These responses of TSH indicated the hypothyroidism, while the value of serum T₃ by radioimmunoassay was quite normal. Therefore it was interpreted that there was compensatory release of TSH which was caused by the feedback mechanism in response to the low serum T₄ level and that in consequence of this, a quite normal level of serum T₃ was maintained, rendering the patient’s physical and mental development to proceed normally. Similar serum patterns of T₄, T₃ and TSH in patients with congenital goiter (Chopra et al. 1975) and post-¹³¹I treated Graves’ disease (Sterling et al. 1971) have been reported.

The administration of TSH produced no further rise in ¹³¹I uptake (Table 1). It was interpreted as indicating a maximum pituitary thyrotropic stimulation. The low level of serum T₄ continued during the administration of TSH. On the other hand an increase in serum level of T₃ occurred, though in a lower degree than in the normal control. It is of interest that T₃ is easier to be secreted than T₄ in response to TSH stimulation. The responsiveness of T₃ to exogenous TSH also suggested that the normal level of serum T₃ in the patient had been maintained by hypersecretion of endogenous TSH.

Analysis of the gland of the patient with Pendred’s syndrome has generally disclosed high MIT/DIT and high iodotyrosine/iodothyronine ratios (Milutinovic et al. 1969). A similar result was obtained in the present study. Ermans et al. (1968) found that, when iodine concentration of the thyroid falls below 200-300 μg/g of wet weight, the MIT/DIT ratio rises and the fraction of iodine in iodothyronine forms falls. Furthermore, Greer et al. (1968) found using perfusion of the thyroid gland, that iodine deficiency resulted in an increase in the T₃/T₄ ratio in the thyroid effluent as well as within the thyroid gland. Since the iodine concentration of our patient was only 60.6 μg/g, we consider that preferential secretion of T₃ was occurring in our patient.
Increased Peroxidase Activity in Pendred’s Syndrome

Normally, iodide trapped into the thyroid gland is oxidized and bound to tyrosyl residues in thyroglobulin molecules. The oxidizing agent is known to be hydrogen peroxide, and the reaction is catalyzed by peroxidase (Taurøg 1970). The patients with familial goiter resulting from an iodide organification defect have been diagnosed by the perchlorate or thiocyanate test. The ability of the anions used in the test to displace the unbound iodide had been considered to be a manifestation of peroxidase deficiency. Recent biochemical studies have confirmed that iodide peroxidase activity was absent in goitrous cretinism (Haddad and Sidbury 1959; Niepomnischce et al. 1973; Valenta et al. 1973) and in patients with goiter, partial perchlorate discharge, euthyroidism and normal hearing (Hagen et al. 1971; Niepomnischce et al. 1972). But Pendred’s syndrome with goiter, euthyroidism, nerve deafness and partial perchlorate discharge was reported to have a normal peroxidase activity (Burrow et al. 1973). An increased peroxidase activity has been reported in an abstract (Ljunggren and Vecchio 1969). In the present study we found an increased amount of iodide peroxidase activity in the gland of our patient with Pendred’s syndrome as compared with controls. One patient with Pendred’s syndrome who had clinical signs of slight hypothyroidism was reported to have a normal peroxidase activity (Ljunggren et al. 1973), but his TSH level was not measured. Nagataki et al. (1973) investigated the changes in peroxidase activity in response to several in vivo manipulations designed to change thyroidal iodine metabolism in rats. It was concluded that TSH is important to maintain peroxidase activity and that a chronic high level of TSH increases the activity. Then it is probable that an increased peroxidase activity in our patient might be related to the increase in serum level of TSH.

The thyroglobulin from the thyroid of our patient appeared to be normal from the results of chromatography on Sephadex G-200 and immunological reaction with antihuman thyroglobulin antibodies.

It was suggested that the patients with Pendred’s syndrome might have a defective hydrogen peroxide producing system rather than the defect of peroxidase activity or defect of the thyroglobulin molecule (Burrow et al. 1973; Ljunggren et al. 1973). However, the activities of superoxide dismutase (McCord and Fridovich 1969) and NADPH cytochrome c reductase (Williams and Kamin 1962), an enzyme suggested to be possibly responsible for hydrogen peroxide production in the thyroid, were not decreased in the thyroid gland of Pendred’s syndrome (Burrow et al. 1973).

Further evaluation is necessary in order to elucidate the cause of intrathyroidal iodide organification defect in Pendred’s syndrome.

Acknowledgments

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References


