Serum thyroglobulin (Tg) stimulation with bovine TSH: a useful test for diagnosis of congenital goitrous hypothyroidism due to defective Tg synthesis

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Abstract. Sixteen patients with congenital goitre were submitted to a bovine TSH stimulation test (bTSH), and serum thyroid hormones (T3, T4), TSH and thyroglobulin (Tg) were measured before and after bTSH injection. In 9 patients with an organification defect basal levels of Tg were normal (19.4 ± 3.1 μg/l) rising after bTSH to a mean level ± SEM of 37.3 ± 4.1 μg/l. Six patients with defective Tg synthesis or release had a mean basal level of serum Tg of 8.7 ± 1.9 μg/l (mean ± SEM) and failed to raise serum Tg concentrations after bTSH (mean ± SEM: 10.4 ± 2.1 μg/l). In both groups a modest although significant (P < 0.05) change in serum thyroid hormones after bTSH was noted. We conclude that the bTSH test may be used to distinguish the group with defective Tg synthesis or release from other types of congenital goitre.

Under physiological conditions, thyroglobulin (Tg), the important precursor protein for synthesis of the thyroid hormones, can be found in the blood in low, but detectable concentrations (Van Herle et al. 1979; Torrigianini et al. 1969; Lo Gerfo et al. 1979; Refetoff & Lever 1983). Although its physiological role outside the thyroid gland is unknown, changes in serum Tg levels occur in a variety of conditions. Elevated levels are noted with increased thyroid gland stimulation (Van Herle et al. 1976; Gardner et al. 1979) and serum Tg may be undetectable in the sera of subjects receiving suppressive doses of thyroid hormone (Mariotti et al. 1982).

Endogenously released TSH (after TRH) or bovine TSH (bTSH) cause Tg release in normal subjects and in goitrous patients (Uller et al. 1973; Unger et al. 1980). Stimulated Tg release depends not only on the magnitude of the injected dose of bTSH but also on basal Tg levels (Unger et al. 1980). Undetectable levels of Tg in plasma in congenital hypothyroidism are associated with the absence of thyroid tissue (Ket et al. 1981; Czernichow et al. 1983) but detectable and variable concentrations of serum Tg were reported in congenital goitre with a genetically induced defect in Tg synthesis or secretion (Gons et al. 1983; Medeiros-Neto et al. 1984). On the other hand, normal or high levels of serum Tg may be found in other inherited disorders of thyroid metabolism such as in the syndrome of organification defect (Gons et al. 1983; Pacini et al. 1984).

In this paper we show that the bovine TSH stimulation test could be useful for distinction between patients with defective Tg synthesis or release and other forms of congenital goitrous hypothyroidism.

Material and Methods

Patients

Sixteen patients with congenital goitre were studied. Ten subjects (4 males, 6 females, ages ranging from 9–28
years) had a positive perchlorate discharge test associated with an early high iodine (131I) uptake, were euthyroid or hypothyroid, had abnormal peroxidase enzyme activity and in vivo iodine kinetics consistent with an expanded thyroidal iodide pool. Most of the clinical and laboratory data of these patients have been reported, in detail, elsewhere (Medeiros-Neto et al. 1979a,b, 1982, 1984). The final diagnosis for this group of patients was a defective, abnormally bound or absent thyroid peroxidase system. Six other subjects (3 males, 3 females, ages ranging from 8–21 years) had congenital goitre, a high iodine uptake test, were hypothyroid or presented with a low thyroid reserve, had an elevated PBI level associated with normal or low T4 and had a negative perchlorate discharge test. Serial serum chromatographies, measuring the relative serum concentration of iodine containing substances following a tracer dose of 131I, were able to demonstrate an abnormally high iodinated protein (iodoalbumin), low levels of iodothyronines (T3 + T4) and only traces of iodothyronines (MIT + DIT). In 5 subjects it was possible to obtain thyroid tissue for in vitro studies. Defective Tg synthesis was confirmed by a very low concentration of Tg/g of tissue (0.0012–0.0133 mg/g of tissue, normal range 60–80 mg/g tissue), no detectable mature Tg (17–19S) on ultracentrifugal studies, and absence of a reactive band with anti-human Tg in immunoelectrophoresis. A clinical and laboratory diagnosis of defective Tg synthesis or release was made, based on these findings.

**Methods**

Tg RIA was performed according to the method of Van Herle et al. (1972) using labelled Tg (125I), and rabbit anti-human Tg antibodies supplied by the NMS Pharmaceuticals Inc., Newport Beach, CA, USA. As second antibody, an excess of goat antirabbit gammaglobulin serum, was used at a dilution of 1:24. The determinations were done in duplicate. The lower limit of detection was 3 µg/l. For the reproducibility of the Tg measurements the coefficient of variation was calculated. For the intra-assay values this coefficient did not exceed 10%, calculated on the basis of values ranging from 10–80 µg/l. All Tg measurements from the same group of patients were performed in one single assay to avoid inter-assay variation. Tg auto-antibodies were undetectable using the Fujirebio kit (Tokyo, Japan). Thyroid hormones and TSH levels were assayed using commercially available kits of RIA (Diagnostic Co., Inc.). The perchlorate discharge test, plasma chromatography of iodinated compounds and thyroid tissue studies were performed as previously reported (Nedeiros-Neto et al. 1979a, bTSH (10 units, Organon Pharmaceutical Co.) was injected im and blood samples drawn before and 24 and 48 h after the bTSH injection (Uller et al. 1973). The highest level of Tg was obtained at 48 h after the stimulation by bTSH and this level was used for all calculations and comparisons between groups. In 27 normal controls the mean serum level of Tg was 11.4 ± 2.6 µg/l rising to a mean ± SEM value of 33.9 ± 5.7 µg/l.

**Table 1.**

Thyroid function studies in congenital goitre after the bTSH stimulation test: results obtained in the peroxidase defect group (organification defect). Basal and peak levels.

<table>
<thead>
<tr>
<th>Patient</th>
<th>T4 (nmol/l)</th>
<th>T3 (nmol/l)</th>
<th>TSH (mU/l)</th>
<th>Tg (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
<td>Basal</td>
<td>Peak</td>
</tr>
<tr>
<td>1 IM</td>
<td>101.3</td>
<td>148.1</td>
<td>1.80</td>
<td>2.43</td>
</tr>
<tr>
<td>2 RM</td>
<td>136.3</td>
<td>215.6</td>
<td>2.61</td>
<td>3.84</td>
</tr>
<tr>
<td>3 AM</td>
<td>146.8</td>
<td>209.1</td>
<td>2.33</td>
<td>3.66</td>
</tr>
<tr>
<td>4 SM</td>
<td>136.4</td>
<td>175.3</td>
<td>2.27</td>
<td>2.81</td>
</tr>
<tr>
<td>5 JM</td>
<td>123.4</td>
<td>220.8</td>
<td>2.35</td>
<td>3.84</td>
</tr>
<tr>
<td>6 AAM</td>
<td>115.6</td>
<td>131.2</td>
<td>2.00</td>
<td>2.03</td>
</tr>
<tr>
<td>7 CM</td>
<td>48.1</td>
<td>61.0</td>
<td>2.88</td>
<td>2.63</td>
</tr>
<tr>
<td>8 BM</td>
<td>41.6</td>
<td>67.5</td>
<td>2.23</td>
<td>1.66</td>
</tr>
<tr>
<td>9 RL</td>
<td>3.9</td>
<td>6.5</td>
<td>0.31</td>
<td>0.32</td>
</tr>
<tr>
<td>10 DFS</td>
<td>139.0</td>
<td>245.5</td>
<td>2.92</td>
<td>6.22</td>
</tr>
</tbody>
</table>

| Mean    | 99.2       | 148.1*     | 2.17       | 2.94*    | 15.9     | 13.3**   | 19.4  | 37.3**  |
| SEM     | 7.0        | 8.9        | 0.86       | 1.26     | 4.8      | 4.6      | 3.1   | 4.1     |

*P < 0.05 (paired t-test). **P < 0.01 (Wilcoxon test).

To convert SI units to conventional units the following conversion factors should be used:

- T4 (µg/dl) = T4 (nmol/l) × 0.077;
- T3 (ng/dl) = T3 (nmol/l) × 65.104;
- TSH (µU/ml) = TSH mU/l;
- Tg (ng/ml) = Tg µg/l.
Table 2.
Thyroid function studies in congenital goitre after bTSH stimulation: results obtained in the thyroglobulin defect group. Basal and peak levels.

<table>
<thead>
<tr>
<th>Patient</th>
<th>T₄ (nmol/l)</th>
<th>T₃ (nmol/l)</th>
<th>TSH (mU/l)</th>
<th>Tg (μg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
<td>Basal</td>
<td>Peak</td>
</tr>
<tr>
<td>1 MRS</td>
<td>6.5</td>
<td>12.9</td>
<td>0.74</td>
<td>0.92</td>
</tr>
<tr>
<td>2 VPM</td>
<td>41.5</td>
<td>50.6</td>
<td>2.01</td>
<td>1.58</td>
</tr>
<tr>
<td>3 CPM</td>
<td>6.5</td>
<td>16.9</td>
<td>0.45</td>
<td>0.74</td>
</tr>
<tr>
<td>4 HCG</td>
<td>79.2</td>
<td>97.4</td>
<td>2.12</td>
<td>3.43</td>
</tr>
<tr>
<td>5 MGL</td>
<td>19.5</td>
<td>35.1</td>
<td>1.33</td>
<td>1.38</td>
</tr>
<tr>
<td>6 RFS</td>
<td>67.5</td>
<td>64.9</td>
<td>3.62</td>
<td>3.73</td>
</tr>
</tbody>
</table>

Mean 36.3 46.7* 1.71 1.96 168.3 156.1 8.7 10.4
± SEM 5.6 5.6 1.07 1.13 10.3 9.9 1.9 2.1

* P < 0.05 (paired t-test).

Results

after the bTSH injection. Student's t-test was employed for testing significance of paired samples (before and after bTSH). As the distribution of TSH and Tg values departed from a normal distribution, a non-parametric test (Wilcoxon test) was used to test the significance between control and bTSH-stimulated values.

The results are summarized in Tables 1 and 2 and represented in Fig. 1. Congenital goitre was not always accompanied by clinical hypothyroidism in both groups of patients. In many patients thyroid hyperplasia and increased thyroid gland function

![Fig. 1.](image-url)

Serum thyroglobulin response to bovine TSH in patients with organification defect (TPO defect) or defective thyroglobulin synthesis (Tg defect).
and secretion were followed by normal serum levels of T4 and T3. A persistent finding was an abnormally exaggerated TSH response to TRH even in the presence of normal serum concentrations of thyroid hormones (Medeiros-Neto et al. 1979b). Basal levels of serum Tg were normal or increased in the peroxidase defect group. After bTSH all patients in this group had a significant increase \( (P < 0.01) \) in serum Tg levels (Fig. 1). This was also followed by rising serum T4 and T3 levels and a significant decrease in serum TSH (Table 1). Serum basal Tg concentrations were normal or at the limit of detection in the defective synthesis or release group (Table 2). None of these patients were able to show an increase in serum Tg after bTSH stimulation (Fig. 1).

**Discussion**

An inherited disorder of thyroid metabolism may be suspected in a child or adult with a goitre from birth, associated with a family history of goitre and/or inbreeding, the presence of overt hypothyroidism or only stunted growth, and laboratory tests indicating high iodide uptake with or without a positive perchlorate discharge test (Medeiros-Neto et al. 1979a, b, 1982; Lever et al. 1983). Further elucidation of the site of the biochemical defect depends upon in vitro tests such as serial chromatography of iodinated serum compounds after a tracer dose of \(^{125}\)I or tests made on material obtained at thyroid biopsy (Lever et al. 1983).

Abnormalities of Tg synthesis and secretion may be related to quantitative or qualitative abnormalities in Tg mRNA or DNA (Von Voorthuizen et al. 1978), the cellular transport of Tg to the apical site of the cell (Lissitzky et al. 1975) or defective glycosylation (Kusakabe 1972). These defects have been described both in animals and man (Lever et al. 1983) and the most important screening test in this group of disorders is simultaneous serum chemical measurement of protein bound iodide (PBI) and thyroxine iodine by radioimmunoassay. Abnormal serum iodoproteins are measured in the PBI but not in the latter. These abnormal iodoproteins may be non-19S Tg subunits iodoalbumin, iodohistidine or iodogammaglobulin (Savoie et al. 1973). Recently Gons et al. (1983) proposed that increased urinary excretion of low molecular weight iodinated material is increased in Tg-deficient patients. This test could be used to allow exclusion of this group of patients from other defective thyroid hormone biosynthesis or other thyroid disorders (Graves' disease, Hashimoto or thyroid carcinoma) because excretion of low molecular weight iodinated material is in the normal range (4–30 µg/g creatinine) in these thyroid conditions. The same investigators mentioned that plasma Tg levels were undetectable in 5 patients whose thyroid glands contained minute amounts of Tg (< 0.05 mg Tg/g tissue) but were in the normal range in 4 patients with a partial defect in Tg synthesis associated with low Tg concentrations in thyroid tissue (1.7–17.0 mg Tg/g tissue). Confirming this finding in our group of 6 patients with defective Tg synthesis or release, serum Tg was detectable in all patients, ranging from 4.0 to 15.3 µg/l. On the other hand 8 subjects in the defective (TPO) group also had normal serum Tg concentrations, ranging from 10.7–27.0 µg/l. Thus basal serum Tg measurements could not distinguish the type of defect in an individual patient. In our group of patients, however, none of the subjects with defective Tg synthesis or release, submitted to a standard bTSH stimulation test, had a significant increase of serum Tg after bovine TSH. Patients with congenital goitre due to an organification defect were able to respond with an increase in serum Tg levels. Thus this test may be of value for an early diagnosis of defective Tg synthesis or release. Final evaluation of the molecular site of the disorder in a particular case, however, will depend on: thyroid biopsy, Tg content in a thyroid extract, electron microscopy for detecting evidence of abnormally rough endoplasmic reticulum membrane and hybridization experiments using DNA complementary to bovine Tg mRNA.

**Acknowledgments**

We are grateful to the expert technical work of Maria Gilda F. Porto. This paper has been supported by a grant from the International Atomic Energy Agency (Vienna, Austria) related to the radioimmunoassay of thyroglobulin (Grant 2900/RB).

**References**


Received on October 15th, 1984.