Hypothyroidism in patients with pseudohyopoparathyroidism type Ia: clinical evidence of resistance to TSH and TRH

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Abstract

Objective: Hypothyroidism is a manifestation of multi-hormonal resistance in pseudohyopoparathyroidism type Ia (PHP Ia). The objective of the study was to determine the mechanisms of hypothyroidism in PHP Ia.

Design: A prospective study.

Patients: Ten patients with PHP Ia.

Measurements: The serum concentrations of TSH, free triiodothyronine (FT₃), free thyroxine (FT₄), and prolactin (PRL) were measured at baseline and after stimulation with TRH (200 µg i.v).

Results: The median basal serum TSH concentration was 4.92 mU/l. Basal serum TSH concentration was slightly elevated in eight patients (4.22–7.0 mU/l; normal range, 0.4–3.6 mU/l), normal in one patient (2.5 mU/l), and high in one patient (13.1 mU/l). After the TRH test, TSH concentrations increased to 13.4–36.0 mU/l (normal range, 4.0–20.0 mU/l). The absolute values after the test were normal in three patients and high in seven patients. However, TSH responses relative to the baseline value (stimulated/basal TSH and expressed as a fold increase), which reflect the relative increases after TRH stimulation, were low in seven patients (2.3- to 4.3-fold TSH) and normal in three patients. Basal FT₄ concentration was normal in seven patients and low in three patients (range, 8.4–20.0 pmol/l; mean, 14.1 ± 4.3 pmol/l; normal range, 10.5–23.0 pmol/l). Basal FT₃ concentration was normal in nine patients and low in one patient (range, 0.9–5.0 pmol/l; mean, 3.8 ± 1.1 pmol/l; normal range, 3.3–6.1 pmol/l). FT₄ and FT₃ were not significantly increased after the TRH test. PRL concentration was normal at baseline and increased from 7 to 96 ng/ml after TRH.

Conclusion: Our results support the hypothesis that patients with PHP Ia have impaired sensitivity to both TSH and TRH.

Introduction

Pseudohyopoparathyroidism type Ia (PHP Ia) is an uncommon genetic disorder characterized by the association between multi-hormonal resistance and clinically abnormal features, called Albright’s hereditary osteodystrophy (AHO), which include short stature, brachydactyly, subcutaneous calcification, obesity, rounded face, and in some cases, mental or developmental abnormalities (1, 2). The genetic defect responsible for the disease is a mutation of guanine nucleotide-binding ⁶-subunit gene (GNAS), the gene encoding the ⁶-subunit of the stimulatory GTP-binding protein. All hormones whose actions are impaired in PHP Ia act through receptors that couple with GNAS, including primarily parathyroid hormone (PTH) in the kidney (1, 2), thyrotropin (TSH) in the thyroid (3–5), and gonadotropins in the gonads (6). Hypothyroidism is one of the main forms of expression of multi-hormonal resistance in PHP Ia and is characterized by high serum TSH concentration, low or normal thyroid hormone concentration, and no goiter. Hypothyroidism is sometimes diagnosed in the neonate (7) or later in life. Because the TSH releasing hormone (TRH) receptor in the pituitary gland is a member of the G-protein-coupled receptor family and can act through GNAS (8), we hypothesized that TRH resistance is another component of multi-hormonal resistance in PHP Ia and that it contributes to hypothyroidism.

The aim of the study was to determine the mechanism underlying hypothyroidism in PHP Ia patients.

Subjects and methods

Patients

Ten consecutive patients with PHP Ia (eight women and two men; age range 25–42 years; median age, 35 years) from four unrelated families were evaluated. All met the
criteria for AHO (short stature, brachymetacarpia, brachymetatarsia, round face, obesity, s.c. calcification, and developmental dental defects), had resistance to PTH (hypocalcemia, hyperphosphatemia, and high levels of immunoreactive PTH), and had low GNAS activity (9). At baseline testing, all were normocalcemic while taking calcium (0.5–1.5 g/day) and vitamin D3 supplements. Out of the ten patients, three had antithyroid antibodies in significant titers (Table 1).

**Determination of erythrocyte GNAS activity**

The biological activity of GNAS was determined using a complementation assay based on the ability of solubilized erythrocyte membrane extracts to restore the responsiveness of adenyl cyclase in membranes prepared from turkey erythrocytes, which lack functional GNAS proteins. The activity in the assay is roughly proportional to the amount of extract GNAS protein added. Heparinized blood samples were collected from patients and control subjects. Soluble extracts were prepared as described by Levine (10, 11). cAMP concentration was measured by a quantitative radioimmunological assay. The results are expressed as a percentage of the activity of a standard membrane preparation comprising pooled erythrocytes from normal subjects and represent the means of triplicate analyses.

**Mutations of GNAS**

Mutations were determined in the four kindred by direct sequencing of the 13 exons of GNAS and exon/intron junctions. The control condition comprised a restriction enzyme when available or a second direct sequencing. Informed consent was obtained from all patients and controls.

**Hormone assays**

Thyroid and lactotropic function were evaluated in all patients before any levothyroxine supplementation therapy. Free triiodothyronine (FT3) and thyroxine (FT4) concentrations were measured using competitive assays with an isotopic tracer (FT3, Diagnost Cis-Biointernational, Gif sur Yvette, France; and FT4, DiaSorin, Antony, France), and TSH was measured by an immunochemiluminometric assay (Immuno-DPC, La Garenne Colombes, France). Prolactin (PRL) concentration was measured by a chemiluminometric assay (Immuno-DPC).

**Provocative tests**

A standard TRH test involving a 200 µg i.v. injection of protirelin (Roche) was performed in the ten patients, and the TSH concentration was measured before the test (T0) and 15, 30, 60, and 120 min after the test. Results are expressed as the basal and stimulated values, and at each time, the absolute increase after TRH (normal, 4–20 mU/l). As advised by Spencer (12), the TSH response relative to the baseline value (stimulated/basal TSH and expressed as a fold increase) was also calculated to reflect the relative increase after TRH stimulation.

Informed consent was obtained from all patients.

**Results**

**Clinical profiles and GNAS activity**

Clinical examination of the ten patients showed evidence of AHO. No patient had clinical evidence of myxedema.

GNAS activity in erythrocytes was about 56% lower than normal (range, 46–62%; mean, 56.3 ± 5.8%). GNAS mutations were identified in the ten patients (Table 2).

**Table 1** Thyrotropin-releasing hormone (TRH) test results for the ten patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>TSH (µU/ml)</th>
<th>PRL (ng/ml)</th>
<th>Antithyroid antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal value</td>
<td>Peak</td>
<td>Fold increase</td>
</tr>
<tr>
<td>1</td>
<td>7</td>
<td>25</td>
<td>3.57</td>
</tr>
<tr>
<td>2</td>
<td>6.44</td>
<td>27.58</td>
<td>4.28</td>
</tr>
<tr>
<td>3</td>
<td>13.06</td>
<td>30.18</td>
<td>2.31</td>
</tr>
<tr>
<td>4</td>
<td>4.22</td>
<td>16.7</td>
<td>3.96</td>
</tr>
<tr>
<td>5</td>
<td>4.26</td>
<td>35.96</td>
<td>8.44</td>
</tr>
<tr>
<td>6</td>
<td>4.92</td>
<td>21.3</td>
<td>4.33</td>
</tr>
<tr>
<td>7</td>
<td>4.54</td>
<td>15.9</td>
<td>3.50</td>
</tr>
<tr>
<td>8</td>
<td>5.97</td>
<td>25.6</td>
<td>4.29</td>
</tr>
<tr>
<td>9</td>
<td>6.08</td>
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<td>5.58</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>13.4</td>
<td>5.36</td>
</tr>
</tbody>
</table>

TPO Ab, thyroperoxydase autoantibodies; TG Ab, thyroglobulin autoantibodies.

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Hormone profiles

The hormone profiles after the TRH test are reported in Tables 1 and 3. Increases in FT3 and FT4 after the TRH test were not measured in patient 6.

The basal TSH concentration was normal in one patient (2.5 mU/l), elevated slightly in eight patients (4.22–7.0 mU/l), and high in one patient (13.0 mU/l). The absolute increase in TSH concentration is shown in Fig. 1a. The fold increase in the TSH response was impaired in seven patients (2.3–4.3) and in the normal range for three patients (5.36–8.44; Fig. 1b), considering 4.4 as the normal lower limit.

The basal FT3 concentration was in the normal range in nine patients and low in one patient. The expected increase in FT3 concentration 3 h after TRH injection was absent in eight patients and moderate in one patient (Fig. 1c).

The basal FT4 concentration was in the normal range in seven patients and slightly low (8.3–10.1 pmol/l) in three patients. The expected FT4 increase 3 h after TRH injection was absent in seven patients and significant in two patients (Fig. 1d).

The basal PRL concentration was in the normal range in nine patients and slightly elevated in one patient (26 ng/ml). Thirty minutes after TRH injection, the PRL response was low in six patients, increased in one patient, and normal in three patients (Fig. 1e).

Discussion

Hypothyroidism was first recognized in patients with PHP Ia in 1971 by Marx and co-workers (13). It is generally mild and involves slightly elevated TSH concentration and normal or slightly low thyroid hormone concentrations. The PHP Ia patients do not have circulating anti-thyroid antibodies and do not develop a goiter (3). The high prevalence of this hormone defect in patients with PHP Ia was underlined in a large series (3); Table 4 presents a review of the literature on thyroid function in patients with PHP Ia.

Hypothyroidism can be associated with other hormone disorders such as hypogonadism, growth hormone deficiency (14), high calcitonin levels (15), and sensorial defects (16), and is attributed to multiple peripheral resistance (3, 16).

The TSH concentration is typically elevated at birth in all newborns with PHP Ia (17–19) and may subsequently normalize for 9–20 months before increasing again. In PHP Ia patients, this could indicate that, like resistance to PTH (9), resistance to TSH progresses during the first 2 years of life. Immediately after birth, there is normally a transient surge in the TSH concentration, which is thought to be a response to the decrease in extracorporeal temperature (20). The exaggerated surge in the TSH concentration at birth in individuals with PHP Ia may indicate a subtle TSH...
resistance that can be detected only during maximal stimulation such as birth. This may lead to recognition of the disease in the neonate (3, 14, 15, 21, 22) or after the first 2 years of life (23).

The mechanisms responsible for hypothyroidism have not been elucidated in individuals with PHP Ia. Several parts of the thyrotropic axis could be involved, as discussed below.

A peripheral mechanism, namely resistance to TSH, is the most common explanation. It is one expression of the signaling defect to polypeptide hormones seen in patients with PHP Ia (3). A series of reports have shown biallelic expression of the GNAS in the thyroid (24–26), but this was associated with preferential maternal expression rather than complete repression of paternal allelic transcription. This relaxed paternal imprinting of GNAS in the human thyroid may be one mechanism responsible for TSH resistance in PHP Ia because of the inactivation of the predominant maternal GNAS allele (23), which would lead to a significant loss of GNAS expression and hormonal resistance. Decreased stimulation of adenylyl cyclase by TSH was reported in thyroid membranes isolated from one PHP Ia patient (27). This mechanism differs from that causing other forms of genetic hypothyroidism with TSH resistance, which comprise the germline mutations of TSH receptor. This defect is characterized by elevated serum TSH and normal or very low serum levels of thyroid hormone in the presence of a hypoplastic or normally sized gland in the proper position in the neck. Depending on the degree of impairment of TSH receptor function, subjects can present with euthyroid hyperthyrotropinemia at one extreme of the spectrum or severe hypothyroidism at the other extreme (28). Indeed, patients can present with mild hypothyroidism with normal thyroid ultrasonography and the absence of thyroid autoantibodies like in PHP Ia, but in this case, hypothyroidism is isolated without specific clinical features like Albright osteodystrophy.

A central mechanism, comprising resistance to TRH, is also considered a possible cause. Although a series of

Figure 1 Stimulation with TRH (200 mg IVD) in ten patients with PSHP Ia. (a) Basal TSH and after TRH in absolute values. (b) TSH after TRH in relative values. (c) Basal FT₃ and after TRH. (d) Basal FT₄ and after TRH. (e) Basal PRL and after TRH.
Table 4  Thyroid function in pseudohypoparathyroidism type Ia (PHP Ia) patients in the literature.

<table>
<thead>
<tr>
<th>Year</th>
<th>Reference</th>
<th>Number of patients</th>
<th>High TSH level</th>
<th>Low FT₄ level</th>
<th>Response to TRH Test &lt;4.4 fold basal TSH</th>
</tr>
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<tr>
<td>1977</td>
<td>41</td>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
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<tr>
<td>1977</td>
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<tr>
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<td>42</td>
<td>1</td>
<td>1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>1990</td>
<td>19</td>
<td>1</td>
<td>1/1</td>
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<td>No test</td>
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<tr>
<td>1991</td>
<td>45</td>
<td>5</td>
<td>4/5</td>
<td>4/5</td>
<td>4/5</td>
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<tr>
<td>1998</td>
<td>37</td>
<td>6</td>
<td>5/6</td>
<td>No data</td>
<td>3/6</td>
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<tr>
<td>1999</td>
<td>23</td>
<td>4</td>
<td>4/4</td>
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<td>7</td>
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<td>1/1</td>
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<tr>
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<td>2/2</td>
<td>2/2</td>
<td>1/2</td>
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<tr>
<td>2005</td>
<td>43</td>
<td>2</td>
<td>2/2</td>
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<tr>
<td>Present study</td>
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<td>9/10</td>
<td>3/10</td>
<td>7/10</td>
<td>Total: 78, Total: 73/78, Total: 16/26, Total: 22/32</td>
</tr>
</tbody>
</table>

reports have indicated that the TRH receptor couple with other G-protein families like GQ and G11 in rat pituitary cell line GH3 (29, 30), a report in which TRH response has been analyzed in GH3 cells has indicated a direct interaction of the TRH receptor with GNAS to cause activation of adenylate cyclase (8). It is thus believed that TRH is able to activate more than one second-messenger system by coupling with its receptor. Little is known about this in human pituitary. The expression of GNAS is imprinted and the transmitting parent determines the variable phenotypic expression of the disease, i.e., PHP Ia when the GNAS mutation is inherited from the maternal allele or pseudopseudohypoparathyroidism type Ia when the mutation is inherited from the paternal allele in individuals with AHO but no hormonal resistance (31). Paternal imprinting in the pituitary gland has been demonstrated recently. GNAS transcription derives mainly from the maternal allele in the pituitary (26, 32), particularly in somatotroph cells, which supports the observation of growth hormone releasing hormone resistance in individuals with PHP Ia (14).

One may hypothesize that TRH resistance is responsible for hypothyroidism in individuals with PHP Ia. The blunted TSH response to exogenous TRH in our study strongly supports this hypothesis. Spencer et al. (12) reported progressively blunted TSH fold responses (4.4 ± 0.2 mean ± S.E.M.) in patients with clinical and subclinical primary hypothyroidism for TSH whose concentration was less than 50 μU/ml, so we considered this value was the lower limit for TSH fold response in Fig. 1b and Table 3. In this last study from Spencer, in patients with normal thyroid function, TSH fold response was 8.5 ± 0.2. De Rosa et al. (33) compared 26 euthyroid healthy subjects and 17 patients with overt or subclinical hypothyroidism using a 200 μg i.v. TRH test. The relative increase in TSH (stimulated TSH/basal TSH) showed constant values in the range of euthyroidism (7.4 ± 2.3, mean ± S.D.), and hypothyroidism (7.7 ± 3.1). This study tends to confirm the data of Spencer (12) showing a relatively constant normal fold TSH response. The values were higher in this last study, but the groups were not exactly comparable with regard to the TSH concentrations. Moreover, the patient groups were larger in Spencer’s study than in De Rosa’s study.

Our study is in agreement with previous reports concerning prevalence of hypothyroidism in PHP, and one of its major interests is the TRH stimulation test data available for the ten patients. Table 3 presents a review of the literature on thyroid function in patients with PHP Ia (7, 19, 22–27, 31–47). Our series shows that hypothyroidism appears to be common in these patients. The response of TSH stimulated with TRH was blunted in most patients in the studies (6, 22, 33, 34, 36–38, 41, 44). However, these studies used different methods for the TRH tests.

Anti-thyroid antibodies were present in patients 1, 2, 5, and 9. Anti-thyroglobulin antibodies were present in one patient, and anti-thyroperoxidase antibodies were high in four patients, all females. These data are available in Table 4. It has been mentioned that anti-thyroid antibodies are present in 10–20% of women, and that TSH increase antibody autoimmunity (48). However, thyroid ultrasonography was performed in all patients and was normal except for patient 5 who had a normal thyroid volume but heterogeneity of the thyroid structure.

The low PRL response to exogenous TRH in our study also supports the mechanism of resistance to TRH. However, PRL secretion also responds to other lactotropic stimuli (49, 50). Sensitivity to TRH, hypocalcemia, and hypothyroidism-modified tonus interfere with PRL production. PRL deficiency has been described in a few studies (3, 36, 45, 51, 52).

A recent report showed that even without the AHO phenotype, PHP Ib is commonly associated with slightly increased basal TSH concentration, which may be related to resistance to TSH (53).
Conclusion

Hypothyroidism is a frequent feature of multi-hormonal resistance in patients with PHP Ia. Hypothyroidism involves resistance to TSH and is related to the defect in expression of GNAS protein in the thyroid. This study has reported TRH test data in a greater number of patients than in previous studies. Our study favors a mechanism of resistance to TRH, in addition to the TSH resistance, which also can be explained by the imprinted expression of GNAS protein in the pituitary.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Acknowledgements

The authors wish to thank Prof. Kottler and Prof. Vassar for genetical analysis, Dr Soudan for prolactin assays, and Dr Basuyau for GNAS activity measurements.

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Received 16 June 2008
Accepted 25 June 2008