Intact parathyroid hormone concentration and cyclic AMP metabolism in thyroid disease

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Abstract. In 35 thyrotoxic patients and 35 patients receiving thyroxine replacement therapy mean serum intact parathyroid hormone concentrations were lower than in euthyroid normal volunteer controls. In 20 hypothyroid patients intact PTH was increased relative to euthyroid controls. Mean serum adjusted calcium was increased in thyrotoxic patients relative to euthyroid controls and in 8 toxic patients with elevated serum adjusted calcium (>2.60 mmol/l) intact PTH was below the assay detection limit (<0.5 pmol/l). Indices of PTH activity were consistent with intact PTH measurements in thyrotoxic patients with nephrogenous cyclic adenosine monophosphate lower, tubular maximum reabsorption of phosphate higher, and urinary calcium creatinine ratio higher than controls. In hypothyroid patients these indices of PTH activity suggest relative end organ resistance to PTH with nephrogenous cyclic adenosine monophosphate similar, tubular maximum reabsorption of phosphate similar, and calcium creatinine ratio lower than in controls. In treated hypothyroid patients nephrogenous cyclic adenosine monophosphate was higher, tubular maximum reabsorption of phosphate similar, and calcium creatinine ratio higher than in controls. These results are compatible with the hypothesis that thyroid status modifies the renal responses to PTH (1-84).

Alterations in calcium and skeletal metabolism are well recognised in patients with thyroid disease. Prolonged hyperthyroidism is associated with negative calcium balance, increased bone turnover, reduced bone density, decalcification and fractures (1-4). Hypothyroidism is characterised by decreased rates of skeletal turnover (2,5), and treatment of hypothyroidism results in a rapid decrease in bone density in some patients (6). The precise mechanisms for these changes are still poorly understood and conflicting evidence exists on the concentrations of immunoreactive parathyroid hormone present and cyclic adenosine monophosphate metabolism in thyroid disease. In hyperthyroidism some studies have demonstrated suppressed immunoreactive PTH (7-9), others have observed no change in immunoreactive PTH concentration (4,10,11) and more recently no change in intact PTH (12). Several groups have reported an increased excretion of urinary cAMP in hyperthyroidism (13,14) and an elevated plasma cAMP (11,15). An elevated urinary cAMP contrasts with suppressed immunoreactive PTH since urinary cAMP is mainly dependent on PTH activity (16,17). However it is more correct to determine nephrogenous cAMP as an index of PTH action at the kidney (18,19) and a study measuring nephrogenous cAMP has found lower concentrations in patients with hyperthyroidism (11).

Some authors have argued that alterations in thyroid status modify the end organ responses to PTH with claims that excess thyroid hormone sensitizes and deficient thyroid hormone blunts the responsiveness of bone to PTH (9) and that hypothyroidism impairs the renal response to PTH (20).

Comparison of previous studies is complicated by the fact that the immunoassays used to measure PTH have been relatively insensitive and of vari-
able specificity. The recent development of immunoradiometric assays (21,22) measuring intact PTH (PTH (1-84)) has improved clinical discrimination in diseases of calcium metabolism and has enabled small changes in PTH (1-84) concentration to be demonstrated even within the reference interval (23). In order to clarify biochemical aspects of calcium metabolism and PTH status in thyroid disease we have studied the relationship between PTH concentrations and indices of PTH activity in patients with thyroid disorders by measuring PTH(1-84) and estimating nephrogenous cAMP, maximal tubular phosphate reabsorption and calcium creatinine ratio in euthyroid controls, thyrotoxic, hypothyroid and treated hypothyroid patients.

Patients and Methods
All patients were attending a thyroid outpatient clinic. We studied 30 euthyroid volunteers (20 female, 10 male, age range 18-65), 35 newly diagnosed hyperthyroid (32 female, 3 male, age range 18-60), 20 hypothyroid (18 female, 2 male, age range 24-80), and 35 treated hypothyroid patients (30 female, 5 male, age range 20-79). Hyperthyroidism, hypothyroidism or euthyroidism was diagnosed following clinical examination, estimation of total thyroxine (TT4), free thyroxine, total triiodothyronine (TT3), thyroid stimulating hormone (TSH), thyroid antibodies and, where appropriate, thyroid scan. All treated hypothyroid patients had received thyroxine replacement therapy for a minimum of 3 months and were considered to be clinically euthyroid with thyroid function tests within the reference ranges for a patient on thyroxine (24).

TT4 and TT3 were measured by an in-house radioimmunoassay using antibodies supplied by the Scottish Antibody Production Unit (SAPU, Law Hospital, Carluke). Free T4 was measured by an in-house radioimmunoassay using magnetic antibody-containing microcapsules (25). TSH was measured by an immunofluorometric assay (Delfia system, Pharmacia). PTH (1-84) was measured in serum using an in-house immunoradiometric assay (22). Plasma cAMP was measured with a commercially available kit (Amersham International plc, Aylesbury, Bucks, UK); urinary cAMP with an in-house method (26) and nephrogenous cAMP calculated by the method of Broadus et al. (19). Serum and urinary calcium, albumin, phosphate and creatinine were measured by standard automated methods. Serum calcium was adjusted for serum albumin concentration. Maximal tubular phosphate reabsorption (TmPO4/GFR) was determined from a standard nomogram.

Statistical analysis was performed using a Kruskal Wallis (ANOVA) test to detect significance for each measurement for the four study groups prior to applying the Mann-Whitney U-test to compare between group significance.

Results
The thyroid function test results for each category of subject studied are given in Table 1. These results confirm biochemical hyperthyroidism, hypothyroidism and euthyroidism. The expected in-

Table 1.
Thyroid function tests.

<table>
<thead>
<tr>
<th>(Reference range)</th>
<th>Euthyroid N=30</th>
<th>Thyrotoxic N=35</th>
<th>Hypothyroid N=20</th>
<th>T4-treated N=35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T4 (55-144)nmol/l</td>
<td>92.5 (16.3)</td>
<td>236.4 (52.8)</td>
<td>41.3 (12.6)</td>
<td>140.6 (28.4)</td>
</tr>
<tr>
<td>Free T4 (8-18)pmol/l</td>
<td>12.8 (1.2)</td>
<td>36.8 (5.3)</td>
<td>5.4 (1.8)</td>
<td>18.1 (3.7)</td>
</tr>
<tr>
<td>Total T3 (0.9-2.8)nmol/l</td>
<td>1.91 (0.23)</td>
<td>5.30 (2.21)</td>
<td>1.40 (0.42)</td>
<td>1.94 (0.24)</td>
</tr>
<tr>
<td>TSH (0.35-5.0) mU/l</td>
<td>1.90 (0.76)</td>
<td>&lt;0.10</td>
<td>41.80 (28.30)</td>
<td>0.95 (1.30)</td>
</tr>
</tbody>
</table>

Figures are mean (sd)
Fig. 1.
PTH(1-84) concentrations in euthyroid healthy volunteers, thyrotoxic, hypothyroid and T₄-treated patients. (PTH(1-84) reference range 1.0-5.0 pmol/l)
Significant differences
p<0.05 Euthyroid vs hypothyroid, euthyroid vs T₄-treated
p<0.001 Euthyroid vs thyrotoxic, thyrotoxic vs hypothyroid

Increased mean TT₄, free T₄ and decreased TSH are observed in patients receiving thyroxine replacement therapy (24).

PTH(1-84) concentrations and nephrogenous cAMP are shown for comparison in the scattergrams (Fig. 1 and 2). The mean PTH(1-84) concentrations and standard deviations for each subject group are: euthyroid 2.5 (1.1), thyrotoxic 1.8 (1.3), hypothyroid 3.8 (2.2), and T₄-treated 2.0 (1.4) pmol/l. Nephrogenous cAMP means and sds are: euthyroid 20.4 (10.6), thyrotoxic 17.6 (12.1), hypothyroid 24.2 (14.0), and T₄-treated 24.9 (12.6) nmol/l GF - Glomerular Filtrate.

Analysis of the PTH(1-84) data using the Kruskal Wallis test (ANOVA) confirmed that significant differences (p<0.001) existed between the four data groups allowing Mann-Whitney comparisons to be made between the groups (legend to Fig. 1). As can be seen PTH(1-84) was undetectable (<0.5 pmol/l) in 10/35 patients with hyperthyroidism and in 8/35 treated hypothyroid patients. PTH(1-84) was elevated in 6/20 patients with hypothyroidism. In contrast, Kruskal Wallis comparison of the nephrogenous cAMP data was significantly different at a lower level (p<0.05) reflecting the wider scatter in this estimation. Mann-Whitney comparisons between the groups are given in the legend to Fig. 2. Nephrogenous cAMP was unde-

Fig. 2.
Nephrogenous (N) cAMP estimations in euthyroid healthy volunteers, thyrotoxic, hypothyroid and T₄-treated patients. (NcAMP reference range 8.0-30.0 nmol/l (GF))
Significant differences
p<0.05 Thyrotoxic vs euthyroid, thyrotoxic vs hypothyroid, thyrotoxic vs T₄-treated

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Table 2.
Biochemical measurements in study groups.

<table>
<thead>
<tr>
<th>(Reference ranges)</th>
<th>Euthyroid N=30</th>
<th>Thyrotoxic N=35</th>
<th>Hypothyroid N=20</th>
<th>T&lt;sub&gt;4&lt;/sub&gt;-treated N=35</th>
<th>Significant differences (Mann-Whitney comparison)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjusted Ca</td>
<td>2.40 (0.09)</td>
<td>2.50 (0.20)</td>
<td>2.39 (0.07)</td>
<td>2.41 (0.08)</td>
<td>p&lt;0.05 Thyrotoxic vs euthyroid Thyrotoxic vs T&lt;sub&gt;4&lt;/sub&gt;-treated</td>
</tr>
<tr>
<td>(2.2-2.6) mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca/Cr (&lt;0.5)</td>
<td>0.40 (0.16)</td>
<td>0.62 (0.37)</td>
<td>0.32 (0.12)</td>
<td>0.52 (0.34)</td>
<td>p&lt;0.01 Hypothyroid vs T&lt;sub&gt;4&lt;/sub&gt;-treated p&lt;0.001 Thyrotoxic vs hypothyroid</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>1.05 (0.12)</td>
<td>1.06 (0.26)</td>
<td>1.02 (0.14)</td>
<td>1.10 (0.18)</td>
<td>NS</td>
</tr>
<tr>
<td>(0.8-1.4) mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TmPO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.95 (0.27)</td>
<td>1.10 (0.34)</td>
<td>0.92 (0.31)</td>
<td>1.06 (0.28)</td>
<td>NS</td>
</tr>
<tr>
<td>(0.8-1.35) mmol/l GF</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Plasma cAMP</td>
<td>19.0 (5.5)</td>
<td>28.7 (8.3)</td>
<td>16.8 (3.9)</td>
<td>23.2 (4.8)</td>
<td>p&lt;0.05 Euthyroid vs T&lt;sub&gt;4&lt;/sub&gt;-treated p&lt;0.001 Thyrotoxic vs euthyroid Thyrotoxic vs hypothyroid Thyrotoxic vs T&lt;sub&gt;4&lt;/sub&gt;-treated Hypothyroid vs T&lt;sub&gt;4&lt;/sub&gt;-treated</td>
</tr>
<tr>
<td>(15-30) nmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary cAMP</td>
<td>43.8 (11.2)</td>
<td>48.4 (14.7)</td>
<td>42.6 (13.6)</td>
<td>48.8 (14.8)</td>
<td>NS</td>
</tr>
<tr>
<td>(26-66) nmol/l GF</td>
<td></td>
<td></td>
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</table>

Values are mean (SD)

Detectable in 6/35 hyperthyroid patients and in 2/35 treated hypothyroid patients. In hypothyroid patients nephrogenous cAMP was lower than would be predicted by the respective PTH(1-84) concentrations with 2/20 patients having elevated nephrogenous cAMP.

Table 2 shows the values obtained for adjusted Ca, calcium creatinine ratio (Ca/Cr ratio), serum phosphate (PO<sub>4</sub>), TmPO<sub>4</sub>/GFR, plasma cAMP and urinary cAMP in the subjects studied. The Kruskal Wallis test was significant for adjusted Ca (p<0.05), Ca/Cr ratio (p<0.01), and plasma cAMP (p<0.001). Significant differences for these measurements are given for Mann-Whitney comparison between thyrotoxic, hypothyroid, treated hypothyroid patients or euthyroid controls. Hypercalcemia was present in 23% of patients with thyrotoxicosis. Intact PTH was undetectable in these 8 patients. All treated hypothyroid patients were normocalcemic.

Discussion

Measurement of serum intact PTH concentrations overcomes the sensitivity problems experienced in earlier studies of PTH in thyroid disease. The major conclusion from the results is that a strongly significant difference in intact PTH concentrations exists in patients of differing thyroid status, with intact PTH often decreased in thyrotoxicosis, decreased in treated hypothyroidism, and increased in hypothyroidism. We have confirmed some of the findings of a previous study (12), but in this study a higher percentage of thyrotoxic patients had undetectable intact PTH concentrations. This may reflect differences in patient selection since all our thyrotoxic patients were newly diagnosed and untreated.

In thyrotoxic patients the relative decrease in intact PTH would appear to be a physiological response to an increase in mean serum adjusted Ca.
Eight thyrotoxic patients with intact PTH values below our detection limit were hypercalcemic (adjusted Ca>2.6 mmol/l) and the mean adjusted Ca level was increased relative to controls in thyrotoxicosis. This increase in serum Ca is probably due to Ca release from bone as a result of direct effects of endogenous thyroid hormones on skeletal metabolism. Intact PTH is the active circulating form of PTH and in thyrotoxic patients indices of PTH activity are consistent with decreased serum intact PTH concentrations: nephrogenous cAMP is decreased, TmPO4/GFR is higher, and Ca/Cr ratio is elevated.

In hypothyroid and treated hypothyroid patients the correlation between intact PTH and indices of PTH activity is weaker. Hypothyroid patients have relatively higher intact PTH levels than euthyroid controls and this is associated with normocalcemia. Nephrogenous cAMP is no different, TmPO4/GFR is similar, and Ca/Cr ratios lower than normal, which indicates that there is end organ resistance, at the kidney, to intact PTH in some hypothyroid patients. Impaired responses to exogenous PTH have been demonstrated in hypothyroid patients (9,20), and the reasons for this may be thyroid hormone action on adenylate cyclase activity (27,28) and cAMP phosphodiesterase activity (27,29,30).

Hypothyroid animals have decreased adenylate cyclase activity in the renal medulla, diminished responsiveness to PTH in the renal cortex with either increased or normal phosphodiesterase activity (hepatocyte, adipocyte and renal), which results in decreased cAMP production. It is likely that PTH production is affected similarly in human cells and this may explain the lower plasma cAMP results and the increased intact PTH observed in hypothyroid patients but absence of elevated nephrogenous cAMP.

Hypothyroid patients on thyroxine have a mean intact PTH concentration that is lower than in normals. This is associated with a relatively increased nephrogenous cAMP, a similar TmPO4/GFR, a similar Ca/Cr ratio, and normocalcemia. Our results would indicate that at the kidney, although intact PTH is lower than normal, cAMP production is increased. This may be due to an increase in adenylate cyclase activity and a decrease in cAMP phosphodiesterase activity, as has been observed in hepatocytes and renal cells, when hypothyroid rats were treated with thyroxine (27). The cAMP responses we have observed, increased plasma cAMP, and relatively increased nephrogenous cAMP, are therefore likely to be secondary to thyroxine therapy and the decreased intact PTH concentrations a consequence of thyroxine action on skeletal metabolism.

Our observations have demonstrated the significant changes in intact PTH concentrations that occur in thyroid disease and when taken in conjunction with indices of PTH activity may help to explain the mechanisms involved in calcium metabolism in these patients. Treated hypothyroid patients have subtle differences from euthyroid controls, whether these differences are the result of "over-replacement" with thyroid hormones or simply a consequence of exogenous thyroxine therapy will require further investigation.

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References


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