Serum vitamin D metabolites and calcitriol receptor concentration in parathyroid tissue in primary hyperparathyroidism

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Vitamin D metabolites in serum and calcitriol receptor concentration in parathyroid tissue were examined in 52 patients operated on for primary hyperparathyroidism. The calcitriol receptor levels were not different in parathyroid adenomas (mean 224 fmol/mg of protein, range 29–509, N = 43), normal parathyroid tissue (mean 245, range 31–690, N = 20), and primary parathyroid hyperplasia (mean 172, range 46–477, N = 9). Preoperative serum levels of calcitriol concentration correlated inversely to the calcitriol receptor in normal parathyroid tissue in patients with adenomas (r = -0.57, N = 17, p = 0.017), but no such correlation was found in the corresponding adenomas (r = 0.14, p = 0.59). In 31 patients in whom both pre- and postoperative vitamin D metabolite analyses were carried out, 23 had lower calcitriol postoperative concentrations compared to preoperative values (p = 0.012, sign test). No change was found in the other vitamin D metabolites postoperatively. By multiple regression analysis calcitriol concentration in serum was inversely correlated to the serum concentration of urea and phosphate (p = 0.003). We conclude that calcitriol may influence calcitriol receptor expression in normal parathyroid tissue, but not in adenomatous parathyroid gland. Furthermore, serum calcitriol was correlated with the renal function, and phosphate level, and in most patients the calcitriol concentration was lower after the operation.

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The active vitamin D metabolite calcitriol regulates mineral homeostasis by acting on target organs such as intestine, bone and kidney (1). Calcitriol acts on its target tissues by binding to the calcitriol receptor, a 50–60 kDa protein which has been cloned and shown to be structurally similar to other steroid receptors (1).

Calcitriol receptor was demonstrated in human parathyroid adenomas several years ago, and a possible involvement of calcitriol in the regulation of PTH expression and synthesis was suggested (2). Recent studies have demonstrated an inhibitory effect of calcitriol on the PTH secretion which may have clinical significance (3). Thus, calcitriol has been shown to reduce the concentration of preproPTH mRNA and suppress PTH secretion in bovine parathyroid cells (4, 5). Furthermore, a suppression of PTH gene transcription by calcitriol was found in vivo in rats (6).

Previously, a positive correlation between calcitriol concentration in serum and calcitriol receptor content in the parathyroid glands has been demonstrated in normal and uremic dogs (7). In humans, reduced binding of [3H]calcitriol has been demonstrated in hyperplastic parathyroid glands from patients with renal failure compared to adenomas from patients with primary hyperparathyroidism (HPT) (8). The findings suggest that lower calcitriol receptor levels are involved in the pathogenesis of secondary HPT, and that lower calcitriol receptor concentration renders the glands less responsive to calcitriol in chronic renal disease (7, 8). Recently, the effect of an acute injection of calcitriol on calcitriol receptor concentration was studied in vivo in rats (9). It was found that injection of calcitriol resulted in up-regulation of calcitriol receptor mRNA and a reduction of PTH mRNA.

In this study we examined the calcitriol receptor concentration in adenomas, hyperplasia and in normally appearing parathyroid tissue from patients who were operated on for primary sporadic HPT. Calcitriol receptor analysis in normal parathyroid tissue in humans has not to our knowledge been reported previously. We also examined the relationship of the calcitriol receptor to the vitamin D metabolites in serum.

Materials and methods

The patients

This study is based on data from 52 patients with a mean age of 64 years (21–85 years, 11 M, 41 F) operated on for primary HPT from April 1986 to February 1989. All patients were hypercalcemic and with the diagnosis confirmed at the operation. The surgical strategy aimed
at identifying all parathyroid glands. In cases with one parathyroid gland enlarged and normal appearance (size and colour) of the others (N = 43), usually the adenoma plus the ipsilateral normally-sized gland were removed for histological examination. In cases with more than one gland enlarged (N = 9), 3 to 3½ glands were removed. If the patient had multiple gland disease peroperatively, the clinical diagnosis was considered as being hyperplasia of the parathyroid glands. Otherwise the diagnosis in this study was classified as adenoma (single gland disease). A sample from each surgical specimen was trimmed and snap-frozen in liquid nitrogen and stored at −70°C. The main part of the specimen was used for histological examination. In 7 out of 40 patients with adenoma (slides not available in 3 cases), hyperplasia was suggested by histological evaluation. The histology of biopsies from patients with hyperplasia always supported the peroperative diagnosis (slides not available in two patients). As no definite criteria exist to differentiate hyperplasia from adenoma (10), the results of the diagnostic evaluation made peroperatively were used.

Postoperative vitamin D metabolite serum samples were taken when calcium concentration in serum was normalized, or before discharge from the hospital (median = day 4, range = day 1−15). Forty-nine patients became normocalcemic after surgery, whereas three patients, all with parathyroid hyperplasia, remained hypercalcemic.

The calcitriol receptor analysis

The frozen parathyroid tissue samples were minced and homogenized using an Ultra-Turrax homogenizer (Janke and Kunkel, Ika-Werk, Staufen, Germany) in ice-cold KTE buffer [KCl 0.3 M, TRIS-HCl 20 mmol/l, EDTA 1 mmol/l, Na₂MoO₄ 10 mmol/l, dithiothreitol 2 mmol/l (Bio-Rad Laboratories, Richmond, CA), and Trasylol 10⁶ kallikrein inhibitor units/l (Bayer, Leverkusen, Germany), pH = 7.4], using 75 ml/g wet weight, or at least 1 ml of buffer, and ultracentrifuged at 180,000 g for 60 min (Beckman L-8 55). The tissue extracts were always kept at 0−4°C. For the saturation binding assay and the Scatchard analysis, 200 μl supernatant was incubated with 5 μl of increasing concentrations of [³H]calcitriol (Amersham International, Buckinghamshire, UK) (18−323 pmol/l, final concentration) on ice overnight. For the one-point calcitriol receptor analysis, 200 μl supernatant was incubated with 5 μl of 323 pmol/l (final concentration) [³H]calcitriol. Non-specifically bound tracer was determined by incubating the supernatant with tracer and 200-fold more non-radioactive ligand. Bound and free tracer was separated by the hydroxylapatite method (11).

The protein concentration was determined by the Coomassie blue dye method (12) using bovine serum albumin as standard. The DNA concentration was determined in hydrolyzed pellets from the centrifugation of the homogenate (13). The calcitriol receptor concentration was measured as fmol/mg of protein, or as otherwise stated.

The analysis of the serum samples

The vitamin D metabolites were analysed as previously described (14−16). PTH was measured by using a mid-molecule PTH assay (from immuno Nuclear, Stillwater, MN). The inter- and intra-assay coefficients of variation of the mid-molecule PTH assay were 5.1% and 6.7%, respectively. Recently, an intact PTH assay was also used (Allegro intact PTH, Nichols Institute, San Juan Capistrato, CA). The other biochemical analyses were measured as part of the routine at the laboratory (Chem 1, Technicon. Courtesy of the Laboratory of Clinical Biochemistry, Haukeland Hospital).

Statistical analysis

Non-parametric tests were used for the comparison of the calcitriol receptor levels between groups, pre- and postoperative serum concentrations (two-sided tests), and for estimation of correlation coefficients (Spearman rank correlation). Linear multiple regression analysis was used for determining the regression coefficients.

Results

The calcitriol receptor analysis

In this study, the one-point receptor assay was highly correlated to the maximal binding capacity found by Scatchard plot analysis (r = 0.98, N = 59, p < 0.001). The calcitriol receptor concentration was considered as being the binding capacity of the one-point receptor assay multiplied by 1.35 according to least-square regression analysis. The dissociation constants examined by Scatchard plot analysis varied between 16 and 143 pmol/l (mean = 56 pmol/l, sd = 32, N = 59).

Control experiments of the calcitriol receptor assays were performed using the one-point calcitriol receptor analysis. Ten adenoma samples were divided in two specimens (weight of specimens, mean = 118.6 mg, sd = 86.5) and analysed in separate assays. The average deviation from the mean of the duplicate calcitriol receptor measures was ±13.2% (SD = 7.3). Furthermore, also the calcitriol receptor concentration in small specimens of adenomas was compared to that in the larger sample (N = 13). By comparing the calcitriol receptor concentration in the smaller (weight, mean = 10.2 mg, sd = 6.1) and the larger samples (weight, mean = 54.7 mg, sd = 26.8) of an adenoma, average deviation from the mean of the duplicates was ±17.8% (sd = 17.1). Overall, the small specimens had 14.4% lower calcitriol receptor concentration compared to the larger samples. The coefficient of variation of the calcitriol receptor concentration in frozen aliquots of
Calcitriol

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(B)

A

human

receptor

serum

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Fig. 4

4-10

Calcitriol

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examined.

Linear

regression (line) was performed (A) of logarithmically transformed calcitriol receptor concentration on the serum calcitriol level.

human kidney tissue extract was 12.8%, N = 19. A high correlation between value of weight, protein and DNA content in the tumours was found (Fig. 1).

Calcitriol receptor examination in the parathyroid glands

A sufficient amount of normal parathyroid tissue was available for calcitriol receptor analysis in 20 patients and, of these, 17 patients had preoperative serum vitamin D metabolites analysed. The serum calcitriol concentrations correlated inversely to calcitriol receptor levels in normal parathyroid tissue (r = -0.57, N = 17, p=0.017, Fig. 2A). No such correlation was found in the corresponding adenomas (r = 0.14, N = 17, p=0.59, Fig. 2B). The relationship was confirmed by relating the calcitriol concentration to the calcitriol receptor levels relative to DNA content or tissue wet weight (r = -0.68, r = -0.61; p=0.003, p=0.009, respectively). In all adenomas examined, the correlation coefficients of calcitriol concentrations and the calcitriol receptor levels related to mg of protein, µg of DNA, and mg of tissue weight were r = 0.15, r = 0.27, r = 0.20, respectively (N = 35).

We could not demonstrate a significant difference between calcitriol receptor concentrations in adenomas,

Table 1. The calcitriol receptor in parathyroid tissue. Maximal binding capacity was examined in samples from 52 patients operated on for primary hyperparathyroidism. In patients with hyperplasia the calcitriol receptor concentration was determined as mean value of the specimens examined in each patient.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>fmol/mg of protein</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenoma</td>
<td>43</td>
<td>224</td>
<td>140</td>
<td>29-509</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>20</td>
<td>245a</td>
<td>171</td>
<td>31-690</td>
<td></td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>9</td>
<td>172b</td>
<td>135</td>
<td>46-477</td>
<td></td>
</tr>
</tbody>
</table>

a Not significantly different from calcitriol receptor concentration in adenomatous tissue. (Wilcoxon's signed rank test.)
b Not significantly different from the calcitriol receptor concentrations in adenomatous or normal tissue. (Rank sum test.)
Table 2. Blood chemistry before and after surgery. Pre- and postoperative serum parameters were analysed. The postoperative samples were taken after normalization of calcium, or before discharge (median = fourth day). Sufficient amount of serum was not available for analysis of all parameters in some patients. Pre- and postoperative values were compared using Wilcoxon's signed rank test.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>sd</td>
</tr>
<tr>
<td>Calcitriol (pmol/l)</td>
<td>43</td>
<td>104.4</td>
<td>48.7</td>
</tr>
<tr>
<td>24,25-DHCC* (nmol/l)</td>
<td>38</td>
<td>2.36</td>
<td>1.98</td>
</tr>
<tr>
<td>25,26-DHCC* (nmol/l)</td>
<td>38</td>
<td>0.84</td>
<td>0.51</td>
</tr>
<tr>
<td>Calcidiol (nmol/l)</td>
<td>43</td>
<td>49.5</td>
<td>23.1</td>
</tr>
<tr>
<td>Chloride (mmol/l)</td>
<td>52</td>
<td>108.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>52</td>
<td>2.97</td>
<td>0.35</td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>52</td>
<td>0.83</td>
<td>0.18</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>52</td>
<td>92.4</td>
<td>30.3</td>
</tr>
<tr>
<td>Urea (mmol/l)</td>
<td>51</td>
<td>6.50</td>
<td>2.95</td>
</tr>
</tbody>
</table>

* DHCC: dihydroxycholecalficrol.

normal and hyperplastic parathyroid tissue regardless of whether the calcitriol receptor concentration was related to mg of protein (Table 1), µg of DNA or mg wet weight.

Serum chemistry

In 36 out of 43 adenomas, wet weight of the total adenomatous gland was determined. The weight of the adenomas was correlated to calcium and PTH concentrations (r = 0.45, p = 0.006; r = 0.47, p = 0.004, respectively), whereas there was no relationship to the phosphate concentration in serum (r = −0.20).

Preoperatively, 8 patients had low calcidiol (<30 nmol/l) and 5 patients had low calcitriol levels (<50 pmol/l) in the serum. In 23 of 31 patients, calcitriol concentrations decreased in serum postoperatively (p = 0.012, sign test) (Table 2, Fig. 3), but the other vitamin D metabolites remained unchanged, including the ratios of 24,25-dihydroxycholecalficrol/calcidiol and 25,26-dihydroxycholecalficrol/calcidiol. Pre- and postoperative calcitriol, and also pre- and postoperative calcidiol levels were correlated (r = 0.48, p = 0.006; r = 0.59, p < 0.001, respectively). The calcitriol concentration was inversely correlated to the level of urea, and phosphate as expressed by multiple linear regression. [calcitriol] = 233 − 7.9 × [urea] − 99 × [phosphate] (p = 0.017) − 99 × [phosphate] (p = 0.04); p = 0.003, N = 42. Though urea and creatinine were significantly correlated (r = 0.62, N = 51), urea explained the variation in calcitriol concentration better than creatinine. Independent correlation coefficients between calcitriol and urea, and phosphate, were r = −0.35 (p = 0.024) and r = −0.34 (p = 0.026), respectively.

The mean preoperative PTH level was 204 pmol/l (range 70–2010, reference range 20–80). The intact PTH assay is now routinely used for measuring PTH in patients suspected of having primary HPT. In these patients a good correlation between the values of mid-region PTH and intact PTH assay was found (r = 0.78, N = 28, p < 0.001).

Discussion

The calcitriol receptor concentration in the small parathyroid samples was measured by using a one-point receptor assay, which gave a good estimate of the maximal binding capacity as judged by the Scatchard analysis. The dissociation constants were similar to that previously reported in human tissue (1). The incubation of tracer and calcitriol receptor was performed on ice.
overnight. This procedure estimated the unoccupied calcitriol receptor level.

In vitro examination has shown that calcitriol inhibits preproPTH mRNA expression and PTH secretion in dispersed bovine parathyroid cells, and also in vivo in rats (4–6), whereas adenomatous parathyroid tissue was resistant to calcitriol (1–10 nmol/l) with no suppression of mRNA levels (4). The levels of calcitriol receptor in bovine parathyroid cells and in human adenomas were similar, suggesting a non-functioning calcitriol receptor in some adenomas (4). The 5'-flanking region of the PTH gene mediates the negative regulation of calcitriol (17). There is evidence of rearrangement in some adenomas in the 5'-flanking region of the PTH gene which may explain the insensitivity to calcitriol in some adenomas (18).

Several studies have demonstrated that the calcitriol receptor level in parathyroid tissue is reduced in animals and humans with renal failure (7, 8, 19), and it has been suggested that the reduced calcitriol binding might contribute to the pathogenesis of secondary HPT. A previous study has demonstrated a positive correlation between calcitriol concentrations in serum and calcitriol receptor in the parathyroid glands (7). The low calcitriol receptor was most evident in uremic animals which also had low calcitriol levels (7, 19). A recent report indicated increased calcitriol receptor degradation in tissues obtained from subtotaly nephrectomized rats (20). It has been suggested that calcitriol might have a role in the up-regulation of calcitriol receptor in the parathyroid glands (7). Thus it has been shown that the calcitriol receptor mRNA was increased 1.7-fold 24 h after an injection of calcitriol to rats (9). It was also shown that the injection of calcitriol potently down-regulated the expression of PTH mRNA. A recent study did not demonstrate up-regulation of calcitriol receptor in parathyroid cell cultures by calcitriol (21).

Our study demonstrated a negative correlation between calcitriol concentration in serum and the calcitriol receptor concentration in normal parathyroid tissue. A similar correlation could not be demonstrated in adenomatous parathyroid tissue whether or not the creatinine concentrations were taken into account. The inverse relationship between serum calcitriol level and calcitriol receptor concentration in normal parathyroid tissue may be due to down-regulation of the receptor or to an increased number of activated receptors not available for binding of tracer. The activated calcitriol receptors induce inhibition of PTH mRNA expression (4, 17). Previously, up-regulation of calcitriol receptor was studied in animals with renal failure or in animals injected with calcitriol (7, 9). Future studies should examine both the expression of PTH mRNA and the regulation of calcitriol receptor and binding of hormone to its receptor in the steady-state situation. The lack of correlation between calcitriol and calcitriol receptor levels in adenomatous parathyroid tissue may account for a non-functioning regulation of calcitriol receptor or calcitriol receptor activation. However, more detailed studies in normal and abnormal parathyroid tissue are necessary to elucidate the molecular regulation by calcitriol.

The level of calcitriol receptor varied much in normal, hyperplastic and adenomatous parathyroid tissue. Receptor destruction may occur, which has been reported using samples from uremic animals (20). In one study of human primary HPT, a lower calcitriol receptor concentration was found compared to what was found in this study (8). A higher calcitriol receptor concentration was found in normal dogs compared to that in this study (7).

In previous studies, the calcitriol concentration in primary HPT has been found increased or not increased compared to reference values (22, 23). In our study, the reduced calcitriol level early postoperatively may be due to a decreased 25-hydroxy-1-alpha-vitamin D3 hydroxylase activity. In a previous study, a reduction of calcitriol was found three months postoperatively accompanied by a significant increase of 24,25-dihydroxycholecalciferol (22). We could not demonstrate a change in either the 24,25-dihydroxycholecalciferol or in the ratio 24,25-dihydroxycholecalciferol/calcidiol in the patients before discharge from hospital.

Several authors have analysed the relationship of calcitriol to other vitamin D metabolites and other parameters preoperatively. They have found that the main determinants for the calcitriol concentration are the concentration of calcitriol and the renal function (24). The study also demonstrated that the calcitriol concentration correlated inversely to calcium, phosphate and PTH levels. It has also been reported that the calcitriol concentration correlated positively to calcidiol, PTH level, glomerular filtration rate, and negatively to calcium and phosphate levels by multiple regression analysis (25).

Employing multiple regression analysis our findings indicated that the calcitriol concentration was inversely related to urea and phosphate. Urea is a parameter of renal function; substitution of urea by creatinine in the multiple regression analysis resulted in a slightly less significant relationship. Low circulating calcitriol levels were prevalent in our patients with primary hyperparathyroidism. However, low calcitriol levels are prevalent in elderly people (26). Although the level of calcitriol was increased in most of our patients with hyperparathyroidism, several patients had low circulating calcitriol despite high levels of PTH. Low circulating calcitriol levels may be due to a high intake of calcium or high circulating calcium concentrations (27, 28). Patients in our study with low calcitriol levels did not have significantly higher calcium levels than patients with normal/high calcitriol concentrations.

A wide spectrum of responses to calcium challenge in patients with primary HPT has been observed. Some patients apparently have essentially autonomous PTH secretion, others show marked suppressibility by cal-
cium of the PTH-calcitriol axis (29). In one reported patient with primary hyperparathyroidism intravenous calcitriol decreased PTH without altering the calcium concentration (30). In another study, intravenous administration of calcitriol was followed by a reduction of serum calcium in six out of nine patients with primary HPT (31). In a few selected cases a role for calcitriol analogues has been proposed in the management of primary HPT (3). Therefore, further studies of the molecular mechanisms in the regulation of the interaction between calcitriol, calcitriol receptor and the parathyroid glands may have an implication for future treatment of the disease.

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References

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