A deficit of calcitriol synthesis may not be the initial factor in the pathogenesis of secondary hyperparathyroidism

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Abstract. Secondary hyperparathyroidism (HPT) develops early in chronic renal failure (CRF) at a time when plasma calcitriol levels are normal. At this time, PTH are higher than normal controls and serum phosphorous levels are lower. A decrement in total serum Ca is noted, after an oral phosphate load, only in patients with ERF. These data suggest that factors, other than a decrease in calcitriol synthesis, may be involved in the pathogenesis of HPT. A hypothesis is forwarded suggesting that an alteration in the newly cloned calcium sensor receptor may be the earliest abnormality in the HPT, preceding a decrease in plasma calcitriol levels.

Key words: calcitrol; calcium sensor receptor; early renal failure; hyperparathyroidism; vitamin D receptor

Introduction

Secondary hyperparathyroidism (HPT) develops early in chronic renal failure (CRF). Both hypocalcaemia and hyperphosphataemia are early factors considered in the pathogenesis of HPT [1,2]. Most of the published observations in patients and in experimental animals have been done in advanced CRF [3-6]. The few available data in patients with early renal failure (ERF) have shown normal serum calcium and phosphorus. In fact, normophosphataemia or mild hypophosphataemia have been consistently observed [7-9]. More recently, a deficit of calcitriol synthesis has been suggested as the major factor in HPT [10]. Such a deficit may lead to a decrease in parathyroid hormone (PTH) inhibition, gut absorption of calcium and worsening of the calcaemic response to PTH. It is likely that changes in serum calcium, phosphorus and calcitriol are all important factors in HPT. However, it is not clear how these factors may interact in ERF. Thus, the seemingly normal blood concentrations of calcium and phosphorus may not reflect actual concentrations of these ions throughout the day. Although serum calcium is usually normal in ERF, it may not be sufficient to inhibit PTH secretion; since it is known that in uraemia a greater serum calcium is needed to inhibit PTH [12,13]. Finally, a major role has been assigned to a deficit of calcitriol as the initial step in the triggering of HPT. However, few data are available in this regard.

The present study evaluates in a large number of patients with CRF the following questions. (1) When in the course of CRF do abnormalities in calcium, phosphorus and calcitriol occur and when does HPT develop? (2) What is the earliest divalent ion abnormality noted in these patients? (3) Since fasting values may not reflect actual changes in divalent ions, what is the effect of an acute phosphate load on divalent ions?

Patients and methods

This study included 165 patients with various degrees of CRF. Of these, 67 were females and 98 were males. Their mean age was 55 years, ranging from 17 to 82 years. Patients excluded from this study included those taking agents such as diuretics, phosphate binders, calcium supplements or vitamin D analogues. Also, patients with a serum albumin less than 3.5 g were excluded. The primary cause of CRF in these patients was as follows: vascular aetiology and/or hypertension was present in 16%; glomerular disease and/or diabetic nephropathy in 40%; interstitial disease and polycystic kidney disease in 28%. The primary cause of CRF could not be established in 16% of the patients.

Patients were distributed into 10 groups according to creatinine clearance (CCR) (Table 1). Thus, group 1 included patients with CCR between 10 and 19 ml/min, group 2 20–29 ml/min and so on. Finally, group 10 included patients with CCR greater than 100 ml/min.

Twenty-eight patients with CCR> 100 ml/min were noted to have PTH values in the upper limit of normal; they were compared with the 14 control subjects with normal renal function and comparable CCR. These patients had a mean age of 42, ranging from 21 to 67 years; 18 were males and 10 females. Their various aetiologies of the renal disease included primary glomerulonephritis (proven by renal biopsy) in 16, renovascular hypertension (proven by arterio-
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Table 1. Mean biochemical parameters of patients, grouped according to creatinine clearances

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>CCR (ml/min)</th>
<th>PTH (pg/ml)</th>
<th>Ctrl (pg/ml)</th>
<th>ICa (mg/dl)</th>
<th>P (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>10-19</td>
<td>164 ± 142**</td>
<td>17 ± 2.6</td>
<td>5.13 ± 0.5</td>
<td>4.23 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>10-29</td>
<td>115 ± 60</td>
<td>18 ± 7.5</td>
<td>4.34 ± 0.3</td>
<td>3.83 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>30-39</td>
<td>115 ± 89**</td>
<td>21 ± 5</td>
<td>4.67 ± 0.3</td>
<td>3.65 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>40-49</td>
<td>69 ± 27**</td>
<td>20 ± 8</td>
<td>4.67 ± 0.2</td>
<td>3.22 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>50-59</td>
<td>52 ± 20</td>
<td>22 ± 9</td>
<td>4.68 ± 0.2</td>
<td>2.89 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>60-69</td>
<td>57 ± 22</td>
<td>23 ± 9</td>
<td>4.69 ± 0.3</td>
<td>2.91 ± 0.5</td>
</tr>
<tr>
<td>7</td>
<td>17</td>
<td>70-79</td>
<td>48 ± 26</td>
<td>22 ± 12</td>
<td>4.79 ± 0.2</td>
<td>3.07 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>80-89</td>
<td>34 ± 20</td>
<td>27 ± 13</td>
<td>4.75 ± 0.3</td>
<td>3.00 ± 0.6</td>
</tr>
<tr>
<td>9</td>
<td>14</td>
<td>90-99</td>
<td>38 ± 21</td>
<td>32 ± 13t</td>
<td>4.72 ± 0.3</td>
<td>3.11 ± 0.5</td>
</tr>
<tr>
<td>10</td>
<td>28</td>
<td>&gt;100</td>
<td>42 ± 13*</td>
<td>31 ± 9†</td>
<td>4.54 ± 0.3</td>
<td>3.05 ± 0.6</td>
</tr>
<tr>
<td>Control</td>
<td>19</td>
<td>&gt;100</td>
<td>28 ± 15</td>
<td>24 ± 9</td>
<td>4.71 ± 0.2</td>
<td>3.08 ± 0.3</td>
</tr>
</tbody>
</table>

*P < 0.013 compared with controls; **P < 0.05 compared with groups 5–10 and controls; †P < 0.05 compared with groups 1–7.

Procedure

Both controls and all patients had blood drawn in fasting conditions for determination of total and ionized calcium, phosphorus, creatinine, calcitriol and PTH. Twenty-four hour urine was collected for calcium, phosphorus and creatinine. Glomerular filtration rate was evaluated using standard CCR corrected for body surface of 1.73 m². Calcium, phosphorus and creatinine were determined with standard autoanalyzer; calcitriol was determined by radioimmunoassay—normal values in our laboratory range between 18 and 78 pg/ml. PTH was determined using a radioimmunometric assay (IRMA) which detects the intact PTH molecule. Normal values in our laboratories are between 15 and 65 pg/ml.

The acute phosphate load was performed as follows: both patients and controls had a phosphate load consisting of regular breakfast containing 400 mg phosphorus, 250 mg of calcium and 350 calories. Immediately after, they had 1 g of elemental phosphorus administered orally at 08.00 h. Prior to breakfast, baseline blood and urinary samples were collected. At the beginning of the study, all subjects had a catheter placed in the cephalic vein for repeated blood sampling. To avoid clotting problems, 1 ml diluted heparin was placed on the catheter. At sampling, the first 10 ml of blood were always discharged to avoid possible contamination with the heparin solution. Prior to the phosphate load and on hourly basis, 6, blood was sampled for determination of creatinine, total calcium and phosphorus. Calcitriol and PTH were determined at baseline and at the end of the 6 h study. Urinary collections were made at 2 h intervals during the study. Urinary determination included creatinine, phosphorus and calcium.

Statistical analysis

For the purpose of comparing the various groups of patients, the ANOVA test was used. To evaluate significant differences between pair groups, the Duncan test was used. Whenever repeated determinations were made in the same patient, the ANOVA test was used [31]. Both bivariant and multivariant analysis were used whenever it was necessary to assess the effect of one variable or to avoid the effect of confounding variables. The paired test was used to test significant differences between paired measurements of the same patients; the P value, when used for multiple comparisons, was adjusted using the Bonferroni method. Linear regression analysis was used to test quantitative associations between two variables. A value of P < 0.05 was considered significant. All the results are expressed as the mean ± SD, except when otherwise indicated.

Results

The mean values of biological parameters of patients distributed in 10 groups according to CCR are displayed in Table 1. Individual values of calcium and phosphorus, as well as calcitriol and PTH, are shown in Figure 1. As can be seen from Table 1, serum ionized calcium was within normal range, while a decrease in serum phosphorus (P < 0.05) was noted in patients with CCR between 50 and 59 ml/min; also, an increment in serum phosphorus was observed in groups 1–3 (P < 0.05). A progressive decrease in calcitriol values was observed as renal function deteriorated (Figure 1C). When groups 9 and 10 (CCR > 90 ml/min) were compared with groups 1–7, a decrement in serum calcitriol was observed (P < 0.05); of interest is that calcitriol levels from groups 8–10 (CCR > 80 ml/min) were higher (P < 0.05) than those from controls.
Fig. 1. Biological parameters of ERF patients. Individual (A) calcium and (B) phosphorus values of all patients are displayed. Mean (C) calcitriol and (D) PTH values from groups according to creatinine clearances are shown; the mid-point of creatinine clearance for each group is displayed in the horizontal axis.

Fig. 2. Individual values of (A) PTH and (B) calcitriol from all patients with CRF, correlated with creatinine clearances.
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A steady and progressive increment in PTH values was noted as renal function worsened (Figure 1D). Values from groups 1–3 were greater than those from groups 4–10 ($P<0.05$). Control subjects had lower PTH values than groups 8–10. Overall, in the 165 patients there was a lineal and inverse correlation between PTH and calcitriol values ($P<0.01$). As shown in Figure 2A, there was a hyperbolic correlation between PTH and CCr ($P<0.001$), and a positive lineal correlation between calcitriol values and CCr ($P<0.001$) (Figure 2B). Similar correlations were observed if the above mentioned parameters were analysed in patients when grouped according to their primary renal disease.

When 28 patients with CCr > 100 ml/min, with seemingly normal PTH, were compared with normal control subjects, significant differences were noted in biochemical parameters (Figure 3). As it can be noted in Figure 3D, these patients have a greater PTH (41 ± 14 pg/ml) than control subjects (28 ± 15 pg/ml; $P<0.01$); this occurred despite the presence of similar calcitriol values in both groups (Figure 3B). Also, mean serum phosphorus was less in these 28 patients (2.9 ± 0.5 mg/dl) compared with normal subjects (3.4 ± 0.4 mg/dl; $P<0.008$).

Biochemical parameters of normal subjects and five patients with early renal failure (mean CCr 73 ml/min) before and after an acute phosphate load are displayed in Figure 4. A progressive linear decrease in serum calcium after the phosphate load was noted in patients (Figure 4A); it decreased from 9.24 ± 0.18 to 8.89 ± 0.28 mg/dl ($P<0.001$). There was a similar linear increment in serum phosphorus in both normals and patients (Figure 4B). Calcitriol values increased significantly from 22 ± 4 to 38 ± 7 pg/ml ($P<0.01$) only in patients (Figure 4C). At this stage of CRF, basal values of calcitriol were less in patients (22 ± 4 pg/ml) than in normals (33 ± 5 pg/ml; $P<0.02$), while basal PTH was greater in patients (40 ± 12 pg/ml) than in controls (19 ± 8 pg/ml; $P<0.01$). The increment in PTH after phosphate load was parallel and of similar magnitude in patients (46 ± 13 pg/ml) and normal subjects (29 ± 7 pg/ml) (Figure 4D). Multivariant analysis for repeated values showed lower PTH values in normal compared with patients ($P<0.01$).

Discussion

The present study demonstrates that patients with CRF have normal serum calcium and phosphorus at various stages of renal insufficiency. Once CCr...
decreases to less than 20 ml/min a change in serum calcium may be detected. Likewise, serum phosphorus was within normal limits and did not increase until CCr was less than 20 ml/min. These observations are in agreement with previous observations [7-9]. Of interest is that patients with CCr between 55 and 65 ml/min had a relative hypophosphataemia. This also is in agreement with previous observations [7-9]. Furthermore, the seemingly normal serum phosphorus observed in patients with GFR > 100 ml/min was in fact lower when compared with control subjects ($P<0.008$). The reason for the seemingly normal or low serum phosphorus may reflect the HPT already present in ERF, which in turn leads to a decrease in TmP leading to phosphaturia. The serum calcitriol was noted to decrease at a CCr < 80 ml/min, when compared with that from patients with CCr > 90 ml/min. However, when the calcitriol values of the former groups were compared with controls, there was no significant difference. The apparent normal calcitriol in patients with CCr > 30 ml/min may be the result of the effect of PTH stimulating calcitriol synthesis; clinically, these observations emphasize the difficulties of interpreting isolated serum calcitriol values in patients with CRF. Surprisingly, calcitriol concentrations in patients with a CCr > 90 ml/min were similar or greater than those in control subjects. The reason for these differences (as we will discuss later) may be the HPT. Thus, mean calcitriol is seemingly normal in ERF patients and it is only later as renal mass decreases that it declines to less than normal.

The changes in PTH observed through the course of CRF followed a different pattern to calcitriol. Thus, in early CRF (CCr > 90 ml/min), at a time when calcitriol is seemingly normal, there was already a significant increase of PTH, which was unchanged until GFR was < 40 ml/min. In fact, the mean PTH was not different from groups 5 to 10. It was only when GFR was less than 40 ml/min that a statistical increase in PTH was noted. The fact that patients with CCr > 100 ml/min have a greater PTH than our normal controls, at a time when calcitriol concentrations were not different from control subjects (Figure 3), suggests that HPT may develop prior to the decrease in calcitriol. This observation militates against a deficit of calcitriol as the initial factor in triggering HPT. Overall, it appears that early HPT may stimulate calcitriol synthesis resulting in seemingly normal calcitriol concentration which, in turn, may temporarily ameliorate the severity of HPT. Once renal mass decrease enough

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**Fig. 4.** Mean values of biological parameters of ERF patients and normal subjects during the acute phosphate load. Normal subjects are represented by an interrupted line; ERF patients by a continuous line. *$P<0.05$, ANOVA test for repeated measurement of each variable.*
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(GFR < 30 ml/min), calcitriol values decline below normal, and a more severe HPT is noted.

It is interesting to speculate on what is the initiating event in stimulating PTH secretions. One possibility may be the presence of an abnormality of the parathyroid cell receptors, either in the vitamin D receptor (VDR) or the recently cloned calcium sensor receptor (CSR) [14]. Earlier, Korkor noted a decrease in VDR density in patients with CRF [15]. In addition, VDR density may be regulated not only by calcitriol but also by dietary calcium intake. Vitamin D-deficient chicks ingesting a high dietary calcium display an upregulation of VDR density, while ingestion of a low calcium diet has the opposite effect [16]. The normal serum calcium and calcitriol seems to mitigate against an early VDR abnormality in ERF. Another possibility may be the presence of an abnormality of the CSR in the parathyroids. As we know, the maintenance of a stable internal environment within complex organisms requires specialized cells that sense changes in extracellular concentrations of specific ions such as calcium. Parathyroid cells possess a cell sensing mechanism that is essential in the regulation of PTH secretion. Though the molecular nature of this sensor has been known, recently an extracellular calcium sensor receptor, BoPCaRI, from bovine parathyroids, has been cloned and characterized [14]. The bovine calcium receptor has a deduced amino acid sequence with a topology similar to the G-protein-coupled receptor superfamily and a large extracellular region with nine glycoxylation sites [14]. Thus, an early abnormality of this receptor may be present in ERF and the seemingly normal serum calcium may not be sufficient to suppress PTH synthesis. Once CCR decreases to less than 80 ml/min, calcitriol decreases and it becomes an important factor in aggravating the HPT.

Finally, the acute phosphate load resulted in a significant decrease in serum calcium only in patients with ERF; this occurred despite a similar increment in serum phosphorus during the acute phosphate load in both groups. Though calcitriol and PTH increased during the acute phosphate load, this was significant only in patients with ERF. Thus, the mild hypocalcaemia noted in ERF patients occurred despite increased calcitriol and PTH; this is consistent with the presence of an intrinsic abnormality in calcium homeostasis which may be independent of the prevailing levels of calcitriol.

In summary, hyperparathyroidism may develop early in CRF before a detectable decrease in CCR. At this point, the PTH values are greater than those noted in normal subjects with similar CCR. Since calcitriol is within normal limits, the mechanism responsible for HPT may be other than a calcitriol deficit. Hypothetically, a uraemic generalized defect of receptors could contribute to HPT. Thus, the recently described BoPCaRI may be involved. This hypothesis is supported by observations of resistance to other hormones, such as growth hormone and insulin, in ERF [17-19]. Once CCR decreases to less than 80 ml/min, a decrease in calcitriol synthesis becomes an additional pathogenic factor in the HPT. PTH is maintained in the upper limit of normal as long as the prevailing concentrations of calcitriol are sufficient to modulate PTH secretion. As CCR decreases to less than 40 ml/min and renal mass decreases, calcitriol decreases leading to worsening of HPT. Furthermore, the ingestion of a high phosphorus diet may unmask a pre-existing abnormality in calcium homeostasis resulting in hypocalcaemia and PTH stimulation, despite prevailing normal calcitriol values.

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References


