Proliferation in hyperplastic human and normal rat parathyroid glands: Role of phosphate, calcitriol, and gender

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Proliferation in hyperplastic human and normal rat parathyroid glands: Role of phosphate, calcitriol, and gender.

Background. Parathyroid gland hyperplasia develops in azotemic patients. A phosphate excess and calcitriol deficiency play critical roles in its development. Our goals were to determine whether differences in serum phosphate values at parathyroidectomy (PTX) in hemodialysis patients with refractory hyperparathyroidism: (1) correlated with parathyroid cell proliferation; and (2) affected the antiproliferative response to in vitro calcitriol. Studies were also performed to determine whether the phosphate concentration in the medium affected the antiproliferative response to calcitriol, and whether a high phosphate diet and calcitriol treatment affected parathyroid cell proliferation and parathyroid hormone (PTH) levels in normal rats.

Methods. Forty-seven parathyroid glands from 19 hemodialysis patients were obtained at PTX. Flow cytometry was used to determine cell proliferation (percent cells in S phase) in excised parathyroid glands. Similarly, cell proliferation was determined in parathyroid tissue incubated for 24 hours in medium with or without $10^{-7}$ mol/L calcitriol and with 1 or 4 mmol/L phosphate. In normal rats, the effect of 3 days of a high phosphate diet (1.2% P) and calcitriol treatment (100 pmol/kg) on PTH values and cell proliferation was evaluated.

Results. In cells from freshly removed parathyroid glands obtained at PTX from hemodialysis patients, there were no significant correlations between the percent cells in S phase and age, gender, and serum phosphate, calcium, and PTH. While incubation of parathyroid tissue with $10^{-7}$ mol/L calcitriol did reduce cell proliferation ($P < 0.001$), both the pre-PTX serum phosphate value ($P = 0.003$) and female gender ($P = 0.003$) were associated with a decreased response to calcitriol. Incubation of parathyroid tissue in medium containing 4 mmol/L phosphate did not increase cell proliferation. In normal rats, a high phosphate diet for 3 days increased cell proliferation ($P < 0.05$) and PTH levels ($P < 0.05$), and calcitriol treatment was without effect.

Conclusion. Our findings suggest a high phosphate burden, as well as female gender, favor parathyroid cell proliferation and both may reduce the inhibition of parathyroid function by calcitriol.

Hyperplasia of the parathyroid gland develops in azotemic patients as chronic renal failure evolves [1-3]. A calcitriol deficiency, hypocalemia, hyperphosphatemia, and skeletal resistance to parathyroid hormone (PTH) are major factors in its pathogenesis [4]. Because calcitriol decreases PTH synthesis and also inhibits parathyroid cell proliferation [5, 6], dialysis patients are treated with calcitriol to control secondary hyperparathyroidism. But a significant number of dialysis patients fail to respond to calcitriol treatment [7-9], which may, in part, be explained by a decrease in vitamin D receptors on parathyroid cells [10]. But calcitriol treatment also fails to control hyperparathyroidism in dialysis patients with hyperphosphatemia [7, 9].

Hyperphosphatemia can increase PTH values by both a direct effect [11-14] and increasing skeletal resistance to PTH [15, 16]. High dietary phosphate, even in the absence of changes in serum calcitriol values, increases parathyroid cell proliferation and stimulates development of parathyroid gland hyperplasia [17, 18]. In azotemic rats, Dusso et al [19] have shown that a high phosphate diet increases parathyroid cell proliferation by increasing transforming growth factor (TGF)-α. Calcitriol is known to act on parathyroid cells through specific receptors for calcitriol [20] and putatively decreases cell proliferation [6] by inhibiting c-myc [21], which then results in stimulation of p21 [22, 23]. Thus, in renal failure, phosphate loading and/or hyperphosphatemia not only act to increase the
demand for PTH, but also directly increase parathyroid cell proliferation.

In the present study, we have evaluated the magnitude of parathyroid cell proliferation present in freshly excised hyperplastic parathyroid glands from hemodialysis patients with refractory hyperparathyroidism. In incubation studies of these same parathyroid glands, we evaluated the in vitro capacity of calcitriol to inhibit parathyroid cell proliferation. Our goal was to determine whether differences in the serum phosphate concentration at parathyroidectomy (1) correlated with parathyroid cell proliferation in freshly excised parathyroid glands, and (2) affected the known antiproliferative response to incubation with calcitriol. Studies were also performed to determine (1) the effect of a high phosphate diet on the inhibition of the parathyroid gland parathyroid cell proliferation by calcitriol in rats and (2) whether a high phosphate concentration increases parathyroid cell proliferation in vitro.

METHODS

Freshly excised hyperplastic parathyroid glands from hemodialysis patients with refractory hyperparathyroidism (47 glands from 19 hemodialysis patients) were studied immediately after parathyroidectomy (PTX). The mean age and duration of dialysis were 50.3 ± 2.8 years (range, 22 to 75 years) and 77 ± 9 months (range, 4 to 220 months). Seven patients were male and 12 were female. None of the patients received calcitriol immediately before PTX. Calcitriol was stopped between 2 weeks and 3 months before PTX, except in 1 or 2 patients in whom the last dose was given between 7 and 14 days before PTX. Because it was not possible to study normal human parathyroid glands, freshly excised parathyroid glands from normal dogs were studied. These parathyroid glands were obtained from euthanized dogs (ages 2 to 8 years) at the City Animal Control Service.

Incubation conditions. Immediately after resection, a piece of parathyroid tissue from each gland was maintained in culture medium at 4°C until the experiments were performed. As previously described, parathyroid tissue slices of approximately 1 mm³ were used [24, 25]. Five to 10 tissue slices from the same parathyroid gland were placed in individual wells (24-well dishes from Nunclon Delta SI, InterMed, Denmark) with constant shaking at 37°C in an incubator with a humid atmosphere. The use of 10 tissue slices was done to ensure that the tissue used in each well was representative of the whole parathyroid gland, which may contain different areas with different growth patterns. The incubation medium was buffered (pH 7.4) and in mmol/L contained: NaCl 125, KCl 5.9, MgCl₂ 0.5, NaH₂PO₄, and Na₂HPO₄ 1 (1:2 ratio), Na-pyruvate 1, glutamine 4, glucose 12, and N-(2-hydroxyethyl) piperazine-N’-2-ethanesulfonic acid (HEPES) 25. Insulin 0.1 IU/mL, bovine serum albumin 0.1%, penicillin G 100 IU/mL, and streptomycin 100 µg/mL were added to the medium. CaCl₂ was added to achieve a final target ionized calcium concentration of 1.25 mmol/L as measured with a selective electrode (634 Ca/pH analyzer, Ciba Corning, Essex, UK). The ionized calcium concentration was measured in each well after the completion of the experiment and confirmed that the ionized calcium concentration did not change. Calcitriol 10⁻⁷ mol/L was used in incubation studies because we previously have shown that this calcitriol concentration reduced parathyroid cell proliferation in hyperplastic parathyroid glands and lower concentrations failed to produce inhibition in the same glands [25]. In some experiments, the phosphate concentration was increased in the medium by adding sodium phosphate until a concentration of 4 mmol/L was achieved. As previously reported, when using a high phosphate concentration in the medium [11, 24], additional calcium needed to be added to the medium to maintain the same 1.25 mmol/L target for ionized calcium concentration.

Parathyroid cell proliferation was assessed by the percent of cells in the S phase of the cell cycle. The cell cycle was analyzed by flow cytometry as previously described [26]. Briefly, clean cell nuclei were obtained by the combined action of the nonionic detergent Nonidet-P40 (St. Louis, MO, USA) and trypsin, followed by treatment with a trypsin inhibitor (type II-O) to stop the trypsin reaction and then by RNAase to prevent dye binding to double-stranded RNA. In a final step, isolated nuclei were stained with propidium iodide and stabilized with spermine. The nuclei were acquired by flow cytometry and analyzed with CELLFIT software (Becton-Dickinson, San Jose, CA, USA) with doublet discrimination module (DDM) to discriminate cell aggregates. This method measures the percent of cells in the different phases of the cell cycle: cells in G0/G1 phase are diploid cells; cells in the S phase show an increase in DNA synthesis that precedes cell duplication; and cells in G2+M have doubled the DNA content or are undergoing mitosis. The percent of cells in the S phase was used as a marker of cell proliferation.

In vivo experiments in rats. Male Wistar rats weighing approximately 200 grams were fed either a normal (0.6%) or high (1.2%) phosphate diet containing 0.6% calcium. All rats ingested 12 g of diet daily and had free access to water. Any rat that did not eat all its food was removed from the study. Half the rats on the normal and high phosphate diets received 100 pmol/kg of intraperitoneal calcitriol daily. This dose was chosen because a previous study showed that it did not increase serum calcium and phosphate values in the normal rat, but did decrease radiothymidine incorporation by parathyroid glands [6]. Rats were sacrificed after 3 days of the experimental diet. Blood was obtained from the aorta, and parathyroid glands were immediately
removed free of thyroid for the analysis of parathyroid cell proliferation.

Reagents. Sodium lauril sarkosinate, proteinase K, phosphate-buffered saline (PBS), RNAase, Triton X-100, DNAase free RNAase, propidium iodide, Nonidet-P40, trypsin, trypsin inhibitor, and spermine were obtained from Sigma Chemical Company (St. Louis, MO, USA) and calcitriol from Abbott, Madrid, Spain.

Statistical analysis. Differences between 2 means were evaluated by the Student t test or the nonparametric Mann-Whitney test. Comparisons of 3 or more means were evaluated by one-way analysis of variance (ANOVA). Linear regression analysis was used to correlate 2 variables, and multiple regression analysis was used to evaluate the correlation between a dependent variable with 2 or more independent variables. Analysis of covariance (ANCOVA) was used to evaluate the effect of several continuous variables (covariates) and that of gender (factor) on parathyroid cell proliferation (response variable). Results are shown as the mean ± SE.

RESULTS

Serum calcium, phosphate, and PTH values at PTX in the 19 hemodialysis patients were 10.85 ± 0.27 mg/dL, 6.26 ± 0.28 mg/dL, and 947 ± 121 pg/mL, respectively. Between the female (N = 12) and male (N = 7) patients, values were not significantly different: 10.85 ± 0.31 versus 10.86 ± 0.53 mg/dL (serum calcium), 6.13 ± 0.32 versus 6.48 ± 0.53 mg/dL (serum phosphate), and 891 ± 141 versus 1044 ± 232 pg/mL (PTH), respectively.

Hyperplastic parathyroid tissue from hemodialysis patients. In the freshly excised parathyroid glands (N = 47), the percent cells in the S phase of the cell cycle, an index of cell proliferation, was 1.1 ± 0.2%. Possible variables which may have affected the percent cells in the S phase were evaluated by multiple regression analysis. Independent variables included serum PTH, calcium, and phosphate values, age, and gender. Results show that none of the independent variables were significant.

Incubation studies of parathyroid tissue from hemodialysis patients. Slices of hyperplastic parathyroid tissue from the 47 parathyroid glands removed from 19 hemodialysis patients were incubated for 24 hours in a medium containing a normal phosphate concentration (1 mmol/L) with or without calcitriol 10^{-7} mol/L. The percent cells in S phase, corrected for the initial S phase value by subtracting the initial from the 24-hour value, was 6.47 ± 0.47% (−calcitriol) versus 3.31 ± 0.60% (+calcitriol), P < 0.001. When calcitriol was absent from the medium, the percent cells in the S phase after 24 hours was similar between females and males, 6.66 ± 0.82 versus 6.00 ± 1.61%, P = 0.69. While calcitriol in the medium for 24 hours reduced the percent cells in S phase in both females and males (P < 0.001), calcitriol incubation also resulted in a lower percent of cells in S phase in males than in females, 4.24 ± 0.80 versus 1.12 ± 0.30%, P < 0.001.

Without calcitriol in the medium, the serum phosphate value present at PTX had a modest but significant correlation (r = 0.29, P < 0.05) with the percent cells in the S phase present after a 24-hour incubation. With calcitriol 10^{-7} mol/L in the medium, the correlation between the pre-PTX serum phosphate value and the percent cells in the S phase after a 24-hour incubation increased to r = 0.42, P = 0.004. The better correlation after incubation with calcitriol occurred at a lower y-intercept as the regression line was displaced downward without a change in the slope. Thus, at serum phosphate values of 6 and 8 mg/dL, the percent cells in S phase were 2.5 ± 0.6% (+calcitriol) versus 5.5 ± 1.2% (−calcitriol) at 6 mg/dL, and 5.0 ± 0.8 ±% (+calcitriol) versus 9.0 ± 2.3% (−calcitriol) at 8 mg/dL.

Shown in Figure 1 is the percent cells in the S phase with and without calcitriol in the medium after the groups were divided by the mean pre-PTX serum phosphate value (6.3 mg/dL). In the absence of calcitriol, the percent cells in the S phase was less (P < 0.05) in the group with the lower serum phosphate value. Moreover, when serum phosphate was less than 6.3 mg/dL, incubation with calcitriol reduced (P < 0.01) the percent cells in the S phase. But when the serum phosphate was greater than 6.3 mg/dL, incubation with calcitriol did not significantly reduce the percent cells in the S phase.

Fig. 1. The effect of pre-PTX serum phosphate values on human parathyroid cell proliferation in vitro. Cell proliferation (% cells in S phase) was determined in parathyroid tissue from 47 glands (from 19 patients) incubated for 24 hours in medium without (dark bars) or with (white bars) 10^{−7} mol/L calcitriol. Groups are divided by the mean pre-PTX serum phosphate value (6.3 mg/dL). Values for percent cells in S phase are given as the mean ± SE. *P < 0.01 vs. (−calcitriol); #P < 0.05 vs. (+calcitriol) of serum phosphate <6.3 mg/dL.
(from male patients) and normal dog parathyroid glands were studied. In both, the percent cells in S phase were not different after a 24-hour incubation, whether a 1 or 4 mmol/L phosphate concentration was used in the incubation medium (Table 3). In parathyroid cells from hyperplastic human and normal dog parathyroid glands, the presence of calcitriol 10^{-7} mol/L in the medium decreased the percent cells in S phase, but again there was not a significant difference between a 1 and 4 mmol/L phosphate concentration.

In vitro effect of high phosphate concentration on parathyroid cell proliferation. To determine whether in vitro exposure to a high phosphate concentration affected parathyroid cell proliferation and the response to calcitriol, parathyroid cells from hyperplastic human

### Table 1. An evaluation by multiple regression analysis of potential factors affecting cell proliferation (percent parathyroid cells in S phase) during a 24-hour incubation with calcitriol

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Standardized Coef</th>
<th>t value</th>
<th>P value</th>
<th>Standardized Coef</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum phosphate</td>
<td>0.44</td>
<td>3.21</td>
<td>0.003</td>
<td>0.42</td>
<td>2.92</td>
<td>0.006</td>
</tr>
<tr>
<td>Serum calcium</td>
<td>0.03</td>
<td>0.24</td>
<td>0.82</td>
<td>0.13</td>
<td>0.88</td>
<td>0.39</td>
</tr>
<tr>
<td>Serum PTH</td>
<td>0.06</td>
<td>0.47</td>
<td>0.64</td>
<td>0.02</td>
<td>0.36</td>
<td>0.72</td>
</tr>
<tr>
<td>Age</td>
<td>-0.07</td>
<td>-0.42</td>
<td>0.67</td>
<td>-0.14</td>
<td>0.73</td>
<td>0.47</td>
</tr>
<tr>
<td>Gender</td>
<td>-0.41</td>
<td>-3.11</td>
<td>0.003</td>
<td>-0.33</td>
<td>-2.31</td>
<td>0.025</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone.

*The percent cells in S phase when calcitriol 10^{-7} mol/L was present in the medium divided by when calcitriol was absent from the medium − overall value is in percent.
*Serum phosphate, calcium, and PTH values are those present at parathyroidectomy.
*Serum phosphate and calcium (mg/dL), PTH (pg/mL), age (years), and gender (female or male).

Multiple regression analysis was used to identify independent variables which could have affected the percent cells in S phase (dependent variable) during the incubation studies. When calcitriol was absent and with age, gender, and serum values of calcium, phosphate, and PTH as independent variables, only serum phosphate at PTX had a marginal effect ($P = 0.08$). When calcitriol was present, the percent cells in S phase correlated both with serum phosphate before PTX ($P = 0.003$) and gender ($P = 0.003$) (Table 1). A similar result was observed when the dependent variable was the percent cells in S phase during calcitriol incubation divided by the percent cells in S phase in the absence of calcitriol. Of the independent variables, again only serum phosphate ($P = 0.006$) and gender ($P = 0.025$) were predictors of the response to calcitriol (Table 1).

ANCOVA was used to better determine the effect of gender. When calcitriol was absent from the medium, the percent cells in S phase (response variable) was neither affected by gender nor by age, the serum calcium, phosphorus, and PTH values present at parathyroidectomy. But as shown in Table 2, when calcitriol was present in the medium, female gender was associated with a higher percent of cells in the S phase. A similar effect of female gender was seen when the percent cells in S phase during calcitriol incubation was divided by the percent cells in S phase observed in the absence of calcitriol (Table 2).

In vitro effect of high phosphate concentration on parathyroid cell proliferation. To determine whether in vitro exposure to a high phosphate concentration affected parathyroid cell proliferation and the response to calcitriol, parathyroid cells from hyperplastic human

### Table 2. An evaluation by ANCOVA to determine whether gender affects cell proliferation (percent parathyroid cells in S phase) during a 24-hour incubation with calcitriol

<table>
<thead>
<tr>
<th>A. Incubation for 24 hours (+calcitriol)</th>
<th>B. Incubation for 24 hours (+calcitriol/−calcitriol)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Response variable</strong>—percent parathyroid cells in S phase</td>
<td><strong>A. Incubation for 24 hours (+calcitriol)</strong></td>
</tr>
<tr>
<td><strong>Covariates$^b$</strong></td>
<td>F ratio</td>
</tr>
<tr>
<td>X—Serum phosphate$^c$</td>
<td>10.29</td>
</tr>
<tr>
<td>X—Serum calcium$^c$</td>
<td>0.04</td>
</tr>
<tr>
<td>X—Serum PTH$^c$</td>
<td>0.13</td>
</tr>
<tr>
<td>X—Age$^c$</td>
<td>0.30</td>
</tr>
<tr>
<td><strong>Factor</strong></td>
<td><strong>Effect of gender$^d$</strong></td>
</tr>
<tr>
<td>All</td>
<td>N</td>
</tr>
<tr>
<td>Female</td>
<td>47</td>
</tr>
<tr>
<td>Male</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>14</td>
</tr>
</tbody>
</table>

ANOVA, analysis of covariance; PTH, parathyroid hormone.

*The percent cells in S phase when calcitriol 10^{-7} mol/L calcitriol was present in the medium divided by when calcitriol was absent from the medium − overall value is in percent.
*Serum phosphate, calcium, and PTH values are those present at parathyroidectomy.
*Serum phosphate and calcium (mg/dL), PTH (pg/mL), age (years), and gender (female or male).
*The mean S phase values in A and B are not the actual means, but rather are mean values adjusted for the average value of the covariate.

### Table 3. In vitro effect of medium phosphate concentration and calcitriol on parathyroid cell proliferation (percent cells in S phase) in parathyroid glands from male hemodialysis patients with severe secondary hyperparathyroidism and from normal dogs$^a$

<table>
<thead>
<tr>
<th>Percent cells in S phase</th>
<th>Without calcitriol</th>
<th>Calcitriol (10^{-7} mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human parathyroid gland (N = 11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (1 mmol/L)</td>
<td>5.3 ± 0.3</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>Phosphate (4 mmol/L)</td>
<td>5.5 ± 0.4</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Dog parathyroid gland (N = 12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (1 mmol/L)</td>
<td>7.4 ± 0.9</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>Phosphate (4 mmol/L)</td>
<td>6.0 ± 1.0</td>
<td>3.4 ± 0.6</td>
</tr>
</tbody>
</table>

Data given are mean ± SE.

*Only parathyroid glands from male hemodialysis patients were used because of the greater calcitriol-induced suppression of parathyroid cell proliferation (percent cells in S phase) in male patients (see Table 2).
*b: $P < 0.001$ vs. without calcitriol; c: $P < 0.05$ vs. without calcitriol.
phosphate diet (NPD) (Table 4). The increase in PTH values in the HPD group was associated with an increase in the percent of parathyroid cells in S phase. Ionized calcium and serum phosphate values were not different between the HPD and NPD groups. Calcitriol administration decreased \( P < 0.05 \) PTH values in rats on a NPD (NPD + calcitriol), but not in rats on a HPD (HPD + calcitriol). As compared to its control on a HPD and on a NPD, calcitriol administration did not decrease the percent cells in S phase. Similar to the difference observed between rats on a HPD and NPD, the percent cells in S phase was greater \( P < 0.05 \) in the HPD + calcitriol group than in the NPD + calcitriol group. Ionized calcium and serum phosphate values were unchanged by the administration of calcitriol.

**DISCUSSION**

We evaluated whether factors present at PTX correlated with the degree of cell proliferation in freshly excised, hyperplastic parathyroid glands. Along with marked elevations in PTH values, hemodialysis patients had hypercalcemia and hyperphosphatemia. Our initial expectation was that the degree of cell proliferation might be affected by the magnitude of hyperparathyroidism, the degree of hyperphosphatemia, or differences in the serum calcium concentration. However, none of these had a significant effect.

In a previous study of freshly excised parathyroid glands from hemodialysis patients with refractory hyperparathyroidism, we showed that calcitriol 10^{-7} \text{ mol/L} in the medium reduced parathyroid cell proliferation during a 24-hour incubation [25]. The present results confirm those findings, but more interesting was that when calcitriol was added to the medium, a high serum phosphate value before PTX adversely affected the response to calcitriol. One possibility is that a high serum phosphate concentration imprints the parathyroid cell with signals that can be detected during the subsequent 24 hours of in vitro incubation and counteracts the suppressive effect of calcitriol.

In incubation studies of parathyroid glands excised from normal dogs and from hemodialysis patients, we showed that increasing the phosphate concentration in the medium from 1 to 4 mmol/L did not increase the percent cells in S phase nor alter the response to calcitriol. These results suggest that imprinting of the signal for parathyroid growth may require more than 24 hours of exposure to a high phosphate concentration or such imprinting can only occur in vivo. The expression of cyclin-dependent kinase inhibitor p21/WAF and the growth promoter TGF-\( \alpha \) has been shown to change in relation to dietary phosphate [19]. Support for our findings about the effect of phosphate also comes from a recent study in which, as in our study, parathyroid cells were cultured from parathyroid glands of patients with secondary hyperparathyroidism [27]. In that study, a high phosphate concentration in the medium increased parathyroid cell proliferation after a long incubation period.

Our results in normal rats and those of others in azotemic rats [17, 18] suggest that dietary phosphate loading can rapidly increase the rate of parathyroid cell proliferation. Moreover, a high phosphate diet was shown to increase parathyroid cell proliferation in azotemic rats by day 2, even before any decrease in the calcium sensing receptor [28]. In our normal rats on a high phosphate diet, ionized calcium and serum phosphate values obtained after 3 days were not different from those in rats on a normal phosphate diet. Even after only 3 days, increased PTH values were accompanied by an increase in parathyroid cell proliferation. Moreover, a calcitriol dose, which was previously shown to decrease (1) radiothymidine incorporation by parathyroid glands of normal and uremic rats [6], and (2) PTH mRNA levels in normal and uremic rats [29], did not significantly reduce PTH values and parathyroid cell proliferation in normal rats on a high phosphate diet. Consequently, our data suggest that dietary phosphate loading not only stimulates PTH secretion, but also induces resistance to the PTH lowering and antiproliferative effects of calcitriol. These results are in agreement with clinical studies showing that calcitriol fails to control secondary hyperparathyroidism in patients with high serum phosphate levels [7–9].

Our study results do not suggest a specific mechanism(s) by which a high phosphate diet increases parathyroid cell proliferation. But Dusso et al [19] reported that a high phosphate diet promoted parathyroid cell proliferation by stimulation of TGF-\( \alpha \). Thus, it is possible that the stimulation of TGF-\( \alpha \) may be important for the imprinting process induced by phosphate.

Because of the known association between the development of primary hyperparathyroidism and female gender [30, 31, 32], previous studies have evaluated whether female gender is associated with more severe secondary hyperparathyroidism in renal failure. Many of these
studies have reported an association between female gender and the magnitude of hyperparathyroidism, the need for parathyroidectomy, the failure to respond to treatment for hyperparathyroidism, or other surrogates of hyperparathyroidism, such as renal osteodystrophy [7, 33–40]. Our own recent experience also supports the association between female gender and the need for parathyroidectomy. Since 1995, we have received parathyroid glands for study from 119 different dialysis patients or renal transplant recipients from several medical centers throughout Spain. Of these 119 patients with refractory secondary hyperparathyroidism, 77 were female and only 42 were male (P < 0.001). Thus, it would seem that just as in primary hyperparathyroidism, female gender is important in the development of secondary hyperparathyroidism. While estrogen has been reported to directly increase PTH gene expression and PTH secretion [41, 42], it has also been suggested that the effect of estrogen could be indirect (e.g., via calcium metabolism) [43]. But if the effect were indirect and estrogen the causal agent, the difference between female and male patients we observed in our ex vivo studies of freshly harvested parathyroid cells would not be expected. Thus, our results provide experimental evidence indicating that differences in gender may affect the PTH response to calcitriol treatment. While we cannot directly attribute this effect to estrogen, it should be considered a likely candidate.

CONCLUSION

Our in vitro results in parathyroid tissue freshly harvested from hemodialysis patients with refractory hyperparathyroidism showed that both female gender and a high serum phosphate value before PTX reduced the antiproliferative effect of calcitriol on cell proliferation. The effect of a high serum phosphate value could not be duplicated by incubation of the parathyroid tissue in a high phosphate medium. In normal rats, a high phosphate diet stimulated parathyroid cell proliferation and prevented the PTH lowering and the antiproliferative effects of calcitriol administration. In conclusion, our findings suggest a high phosphate burden, as well as female gender, favor parathyroid cell proliferation, and both may reduce the inhibition of parathyroid cell proliferation by calcitriol.

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