The In Vitro Effect of Calcitriol on Parathyroid Cell Proliferation and Apoptosis

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Abstract. Calcitriol treatment is used to reduce parathyroid hormone levels in azotemic patients with secondary hyperparathyroidism (HPT). Whether long-term calcitriol administration reduces parathyroid gland size in patients with severe secondary hyperparathyroidism is not clear. The aim of the study was to evaluate in vitro the effect of calcitriol on parathyroid cell proliferation and apoptosis in normal parathyroid glands and in adenomatous and hyperplastic human parathyroid glands. Freshly harvested parathyroid glands from normal dogs and hyperplastic and adenomatous glands from patients with secondary (2°) and primary (1°) HPT undergoing parathyroidec- tomy were studied. Flow cytometry was used to quantify the cell cycle and apoptosis of parathyroid cells. Apoptosis was also evaluated by DNA electrophoresis and light and electron microscopy. In normal dog parathyroid glands, culture with calcitriol (10−10 to 10−7 M) for 24 h produced a dose-dependent inhibitory effect on the progression of cells into the cell cycle and into apoptosis. When glands from patients with 2°HPT were cultured for 24 h, only high calcitriol concentrations (10−7 M) inhibited the progression through the cell cycle and the induction of apoptosis. In parathyroid adenomas (1°HPT), even a high concentration of calcitriol (10−7 M) had no significant effect on the cell cycle or apoptosis. The present study shows that in vitro, calcitriol inhibits in a dose-dependent manner in normal parathyroid glands both parathyroid cell proliferation and apoptosis. However, in secondary hyperplasia, only high concentrations of calcitriol inhibited cell proliferation and apoptosis. In 1°HPT, even high concentrations of calcitriol had no effect. Because calcitriol simultaneously inhibits both cell proliferation and apoptosis, a reduction in the parathyroid gland mass may not occur as a direct effect of calcitriol treatment.

Most dialysis patients develop secondary hyperparathyroidism (2°HPT), which is characterized by high parathyroid hormone (PTH) levels and hyperplastic parathyroid glands. The mechanisms that drive parathyroid cells to proliferate are not clear, but it is generally accepted that a deficiency of calcitriol contributes to the development of parathyroid hyperplasia (1,2). In parathyroid cells, calcitriol acts through the vitamin D receptor (VDR) (3) to inhibit PTH mRNA expression (4,5). Calcitriol has also been shown to inhibit parathyroid cell proliferation (6) by decreasing the expression of the proto-oncogene c-myc (7). In both uremic animals (6) and patients with early renal failure (8), the administration of calcitriol has been shown to prevent the development of 2°HPT. In many patients with fully developed 2°HPT, calcitriol administration reduces the PTH level (9), but patients with severe 2°HPT often fail to respond to calcitriol (10,11). Such a failure may be due to several factors, including a decrease in the number of VDR (12–15) and the presence of hyperphosphatemia (16). In addition, severe 2°HPT is often characterized by the presence of nodular hyperplasia, which may be associated with autonomous monoclonal growth (17).

It is generally accepted that vitamin D compounds are useful in controlling 2°HPT. However, whether calcitriol induces regression of parathyroid gland hyperplasia is controversial (18–20). Apoptosis is the usual mechanism by which cells are deleted from hyperplastic tissues (21). To reduce parathyroid gland size, it would be necessary both to decrease cell proliferation and to increase cell death by apoptosis so that the net result is a decrease in the number of parathyroid cells. The aim of the present study was to evaluate the in vitro effect of calcitriol on parathyroid cell proliferation and apoptosis in freshly harvested normal, adenomatous, and hyperplastic parathyroid glands.

Materials and Methods

The study was performed using freshly excised parathyroid glands from normal dogs and from humans who required parathyroidectomy as a result of primary hyperparathyroidism (1°HPT) and azotemia-induced 2°HPT (12 glands from 12 patients with 1°HPT caused by adenomas and 30 glands from 16 patients with 2°HPT). Immediately
achieve concentrations ranging from 10 to 10
concentration was observed. Calcitriol was added to the medium to
completion of the experiment, and no change in the ionized calcium
calcium concentration of 1.25 mM as measured with a selective
pared with total calcitriol, the concentration of free calcitriol was
by RIA (Gamma 1,25-Dihydroxyvitamin D, IDS, Boldon, UK). As com-
membrane with a 5000-MW cutoff and calcitriol was measured by
the amount of free calcitriol, we filtered the medium through a
penicillin G 100 IU/ml, and streptomycin 100
Incubation Conditions
Experiments were performed using parathyroid tissue slices instead
of dispersed parathyroid cells so that tissue architecture was main-
 Parathyroid glands were cut into small pieces of approximately
mm² and placed in individual wells (24 well dishes from Nunclon
Delta SL, InterMed, Roskilde, Denmark) with constant shaking at
37°C and placed in an incubator with a humid atmosphere. The
incubation medium was buffered (pH 7.4) and contained 125 mM
NaCl, 5.9 mM KCl, 0.5 mM MgCl², 1 mM NaH₂PO₄, and Na₄HPO₄
(1:2 ratio), 1 mM Na-pyruvate, 4 mM glutamine, 12 mM glucose, and
25 mM HEPES. 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 mM as measured with a selective
electrode (634 Ca/pH analyzer, Ciba Corning, Essex, UK). Ionized calcium concentration was also measured in each well after the
completion of the experiment, and no change in the ionized calcium concentration was observed. Calcitriol was added to the medium to
achieve concentrations ranging from 10⁻¹⁰ to 10⁻⁷ M. To quantify the
amount of free calcitriol, we filtered the medium through a membrane with a 5000-MW cutoff and calcitriol was measured by RIA (Gamma 1,25-Dihydroxyvitamin D, IDS, Boldon, UK). As com-
pared with total calcitriol, the concentration of free calcitriol was decreased by one order of magnitude.

Evaluation of Apoptosis
Apoptosis of parathyroid cells was evaluated by DNA electro-
phoresis, electron and light microscopy, and flow cytometry.
DNA Electrophoresis. DNA fragmentation, a characteristic fea-
ture of cell apoptosis, was documented by the electrophoretic separa-
tion of total DNA using a previously described method (22). Briefly,
DNA was isolated after consecutive treatments with proteinase K and
RNAase in a buffer containing 50 mM Tris-HCl, 10 mM ethylenedia-
minetetraacetate, and 0.5% sodium lauril sarcosinate. The electropho-
resis of DNA was performed at a constant voltage of 40 V in
horizontal 2% agarose gels. The bands of DNA were visualized with
ethidium bromide staining.
Light and Electron Microscopy. Light microscopy was used to
identify apoptosis and also to discriminate between nodular and
diffuse hyperplasia. The pieces of parathyroid tissue were fixed in
10% phosphate-buffered saline (PBS)-buffered formaldehyde and em-
bodied in paraffin. Tissue blocks were cut at 5 μ and stained with
hematoxylin and eosin. Apoptosis was also demonstrated by electron
microscopy, for which the tissues were fixed with 2.5% glutaralde-
hyde in 0.1 M phosphate buffer (pH 7.4) and the samples were
embedded in Epon 812 (Fluka, Bunchs, Switzerland); 50-50 nm sections
of tissue were stained with uranyl acetate and lead citrate and exami-
ned with a Philips CM-10 transmission electron microscope (Eind-
hoven, The Netherlands). By light microscopy, the morphologic
changes characteristic of apoptosis included cell shrinkage, nuclear
pyknosis, and fragmentation with formation of apoptotic bodies. By
electron microscopy, changes characteristic of apoptosis were an
intact cell membrane with nuclear changes that included chromatin
condensation and fragmentation. Occasionally, apoptotic bodies sur-
rrounded by a cellular membrane were also present.
Flow Cytometry. Apoptosis was quantified by flow cytometry;
this required isolation of cells that were obtained from the small
pieces of parathyroid tissue used in each experimental test period.
Small pieces of parathyroid tissue were viewed under an inverted
microscope (×10) and were gently stripped apart using sharp Dumont
forceps. This was followed by gentle pipetting. To maintain high cell
concentrations, we performed these manipulations in a small volume
(50 μl) of PBS. Dispersed cells first were treated with PBS containing
0.8% Triton X-100 at 37°C, then with DNAase-free RNAase, 10
μg/ml for 10 min, and finally with propidium iodide, 20 μg/ml for 30
min at 37°C in the dark. Cells were immediately acquired by the flow
cytometer (FACScan, Becton-Dickinson, San Jose, CA). LYSYS II
software (Becton-Dickinson) was used for data acquisition and anal-
ysis. Cell debris and clumps were excluded from analysis by gating.
Apoptotic cells were identified as hypodiploid events that were ob-
erved near the diploid peak.
The cell cycle was analyzed by the method described by Vin-
delov and Christensen (23). Briefly, clean cell nuclei were ob-
tained by the combined action of the nonionic detergent Nonidet-
P40 and trypsin, followed by treatment with a trypsin inhibitor
(type II-O) to stop the trypsin reaction and 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 the flow cytometer and analyzed using
the CELLFIT software (Becton-Dickinson) with doublet discrimi-
nation module to discriminate cell aggregates. This method mea-
sures the percentage of cells in the different phases of the cell
cycle: cells in G0/G1 phase are diploid cells, cells in phase S show
an increase in the synthesis of DNA that precedes cell duplication,
and cells in G2+M have doubled the DNA content or are under-
going mitosis. The percentage of cells in the S phase was used as
a marker of cell proliferation.
Reagents
Reagents included PBS (Oxoid, Hampshire, England) and cal-
citriol (Abbott, Madrid, Spain). Cycloheximide, sodium lauril sar-
cosinate, proteinase K, RNAase, ethidium bromide, Triton X-100,
DNAase-free RNAase, propidium iodide, Nonidet-P40, trypsin,
trypsin inhibitor, and spermine were obtained from Sigma (St.
Louis, MO).
Statistical Analyses
Differences between two means were evaluated by the t test, and
comparisons of three or more means were evaluated by ANOVA
followed by the Duncan test. Regression analysis was used to corre-
late two parameters. Results are expressed as the mean ± SEM.
Results

Normal Dog Parathyroid Tissue

Because normal human parathyroid tissue was not readily available, normal parathyroid tissue from normal dogs was studied.

Cell Cycle and Apoptosis in Normal Parathyroid Cells in Culture. In freshly harvested parathyroid tissue from dogs, there were relatively few cells in the S phase of the cell cycle (0.4 ± 0.1%) and few cells undergoing apoptosis (0.2 ± 0.1%) (Table 1). After a 24-h incubation in a culture medium without calcitriol, the number of cells progressing to the S phase increased to 8.4 ± 1.3% (P < 0.01) and cells undergoing apoptosis increased to 12.4 ± 1.8% (P < 0.01). The analysis by flow cytometry, used to quantify apoptosis, showed the hypodiploid peak (sub G0/G1 peak), which corresponds to cells with DNA fragmentation (Figure 2A). By light microscopy, apoptotic cells were mixed with normal neighboring cells. The electron bodies (Figure 2B). By light microscopy, apoptotic cells were observed by light and electron microscopy. These changes included cell shrinkage, nuclear pyknosis, and fragmentation with formation of apoptotic bodies (Figure 2B). By light microscopy, apoptotic cells were mixed with normal neighboring cells. The electron microscopy (Figure 2C) showed cellular and nuclear shrinkage with intact cell membrane; nuclear changes included chromatin condensation and fragmentation. Occasional apoptotic bodies surrounded by a cellular membrane were also present.

Cell apoptosis is an active process that requires protein synthesis. In a separate set of experiments, the addition of 10 μM cycloheximide, an inhibitor of protein synthesis, resulted in a decrease in apoptotic cells from 12.1 ± 2.7 to 4.7 ± 1.3% (P < 0.01).

Effect of Calcitriol on Cell Cycle and Apoptosis in Normal Parathyroid Tissue. The effect of the addition of calcitriol (from 10⁻¹¹ to 10⁻⁷ M) to the culture medium for 24 h is shown in Figure 3, A and B. Even a low calcitriol concentration (10⁻¹¹ and 10⁻¹⁰ M) reduced the progression to the S phase and to apoptosis. As the calcitriol concentration was increased from 10⁻⁹ to 10⁻⁸ M, a further suppression of cells entering the cell cycle and apoptosis was observed. Moreover, a significant correlation (r = 0.95, P < 0.01) was observed at the different calcitriol concentrations between the number of cells in S phase and those undergoing apoptosis.

Hyperplastic Human Parathyroid Tissue

Freshly harvested parathyroid glands from patients with 1°HPT and 2°HPT undergoing parathyroidectomy were studied.

Cell Cycle and Apoptosis in Adenomatous and Hyperplastic Human Parathyroid Tissue in Culture. The data on the cell cycle and apoptosis in the freshly obtained adenomatous and hyperplastic human tissue is summarized in Table 2. In parathyroid adenomas (1°HPT), the proliferative activity was variable but still was significantly greater than in 2°HPT; the percentage of cells in the G0/G1 phase was significantly decreased in 1°HPT as compared with 2°HPT (81.5 ± 5.7% versus 92.0 ± 1.0%, P < 0.05), and the percentage of cells in S phase also tended to be greater in 1°HPT than in 2°HPT (3.0 ± 1.4% versus 1.1 ± 0.3%, P = 0.06). In 2°HPT, the percentage of cells in the G0/G1 phase tended to be less in nodular than in diffuse hyperplasia (90.2 ± 1.5% versus 93.6 ± 1.3%, P = 0.08). In both nodular and diffuse hyperplasia, the percentage of cells in apoptosis was low and there was no difference between the two types of hyperplasia.

In adenomatous and hyperplastic human parathyroid tissue, the results were similar to those in normal parathyroid glands from dogs. A 24-h culture without calcitriol increased entry into the cell cycle. The S phase increased to 4.5 ± 1.8% (P < 0.05) in 1°HPT and to 6.9 ± 1.1% (P < 0.01) in diffuse hyperplasia and to 7.4 ± 1.4% (P < 0.01) in nodular hyperplasia. The entry into the cell cycle was accompanied by a concomitant increase in cells undergoing apoptosis to 9.3 ± 1.4% (P < 0.01) in 1°HPT and in 2°HPT to 12.8 ± 1.7% (P < 0.01) in diffuse hyperplasia and to 11.9 ± 2.1% (P < 0.01) in nodular hyperplasia. A significant correlation was also observed between the percentage of cells in the S phase and those undergoing apoptosis (r = 0.75, P < 0.01).

Effect of Calcitriol on Cell Cycle and Apoptosis in Human Hyperplastic Parathyroid Tissue. Studies were performed in parathyroid glands from patients with 1°HPT and 2°HPT to evaluate the effect of a 24-h culture with calcitriol on the cell cycle and apoptosis. The in vitro response of hyperplastic glands from patients with 2°HPT to calcitriol concentrations ranging from 10⁻¹⁰ to 10⁻⁷ M is shown in Figure 3, C and D. In contrast to the results in normal dog parathyroid glands, only the highest calcitriol concentration (10⁻⁷ M) was able to reduce the percentage of cells in the S phase (6.2 ± 0.7% to 3.8 ± 0.6%, P < 0.01) and this was accompanied by a decrease in the percentage of apoptotic cells (11.4 ± 1.3% to 7.6 ± 1.4%, P < 0.01).

When the in vitro response to calcitriol in parathyroid glands

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**Table 1. Percentage of cells in different phases of the cell cycle and apoptosis in normal dog parathyroid cells**

<table>
<thead>
<tr>
<th></th>
<th>% G0/G1 Phase</th>
<th>% S Phase</th>
<th>% G2 + M Phase</th>
<th>% Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before culture</td>
<td>95.9 ± 0.9</td>
<td>0.4 ± 0.1</td>
<td>3.7 ± 0.4</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>24-h culture</td>
<td>87.4 ± 1.9a</td>
<td>8.4 ± 1.3a</td>
<td>4.2 ± 0.6</td>
<td>12.4 ± 1.8a</td>
</tr>
</tbody>
</table>

*P < 0.01 versus before culture; n = 14.
with nodular versus diffuse hyperplasia was compared, the response to calcitriol was similar in both types of secondary hyperplasia (Figure 4). Only at high calcitriol concentrations (10^{-7} M) was a decrease in cell entry into the S phase and a decrease in cells undergoing apoptosis observed. In parathyroid adenomas (1°HPT), calcitriol concentrations of 10^{-9} and 10^{-7} M had no significant effect on the proportion of cells in the S phase of the cell cycle (4.7 ± 1.8% cells in control versus
5.2 \pm 2.2\% and 4.6 \pm 1.9\% in calcitriol \(10^{-9}\) and \(10^{-7}\) M, respectively) and apoptosis (9.1 \pm 1.2\% in the control versus 6.9 \pm 2.0\% and 7.0 \pm 1.8\% with calcitriol \(10^{-9}\) and \(10^{-7}\) M, respectively).

**Discussion**

The present study evaluates the *in vitro* effect of calcitriol on the cell cycle and apoptosis of cells from normal dog parathyroid glands and from human parathyroid glands with secondary hyperplasia and with primary adenoma. The results of the study show that in normal parathyroid tissue, calcitriol decreases both parathyroid cell proliferation and apoptosis. In parathyroid tissue from humans with 2°HPT, an inhibition of proliferation was observed only at high concentrations of calcitriol. The inhibition of cell proliferation induced by a high calcitriol concentration was also associated with a decrease in cell apoptosis. Cells from parathyroid adenomas (1°HPT) showed no response to calcitriol.

In freshly harvested parathyroid tissue from normal dogs, the percentage of parathyroid cells in the S phase of the cell cycle and those undergoing apoptosis was low. These results are in agreement with previously reported data from normal parathyroid glands (24–27). By contrast, after 24 h of tissue culture without calcitriol, a significant number of cells progressed into the S phase and this was associated with an increase in apoptosis. Furthermore, a significant correlation was observed between the percentage of cells in the S phase and the percentage of apoptotic cells. From this significant correlation, it can be inferred that calcitriol has a dual effect on parathyroid cells, inhibiting both proliferation and apoptosis.

**Table 2.** Cell cycle and apoptosis values (% cells) in parathyroid glands from patients with 1°HPT and 2°HPT

<table>
<thead>
<tr>
<th></th>
<th>% G0/G1 Phase</th>
<th>% S Phase</th>
<th>% G2 + M Phase</th>
<th>% Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1°HPT (n = 12)</td>
<td>81.5 \pm 5.7</td>
<td>3.0 \pm 1.4</td>
<td>15.5 \pm 7.1</td>
<td>0.3 \pm 0.2</td>
</tr>
<tr>
<td>2°HPT (n = 30)</td>
<td>92.0 \pm 1.0</td>
<td>1.1 \pm 0.3</td>
<td>6.9 \pm 0.6</td>
<td>0.3 \pm 0.1</td>
</tr>
<tr>
<td>2°HPT-diffuse (n = 16)</td>
<td>93.6 \pm 1.3\textsuperscript{b}</td>
<td>0.8 \pm 0.4\textsuperscript{b}</td>
<td>5.6 \pm 1.2</td>
<td>0.2 \pm 0.1</td>
</tr>
<tr>
<td>2°HPT-nodular (n = 14)</td>
<td>90.2 \pm 1.5\textsuperscript{b}</td>
<td>1.4 \pm 0.5</td>
<td>8.4 \pm 1.5</td>
<td>0.3 \pm 0.2</td>
</tr>
</tbody>
</table>

\textsuperscript{a} 1°HPT, primary hyperparathyroidism; 2°HPT, secondary hyperparathyroidism; 2°HPT-diffuse, diffuse hyperplasia; 2°HPT-nodular, nodular hyperplasia.

\textsuperscript{b} \(P < 0.05\) versus 1°HPT.
correlation, it may be assumed that cells that enter but do not complete the cell cycle eventually undergo apoptosis. Parathyroid cells, as do many other cell types, tend to proliferate in vitro (24), and it has been proposed that the same stimuli that induce cell activation and proliferation may also lead to apoptosis (28). This phenomenon may be particularly relevant in tissues with low cell turnover such as the parathyroids. It is interesting that cells, such as neurons, that have irreversibly exited the cell cycle will undergo apoptosis in response to the same molecular events that in dividing cell populations lead to proliferation (29). Thus, the relatively high rate of proliferation observed in culture made this in vitro model suitable for determining an effect of calcitriol on parathyroid cell proliferation and apoptosis.

Our results show that in normal parathyroid tissue, the addition of calcitriol to the tissue culture produced an inhibition of cell proliferation and of apoptosis. This effect of calcitriol was dose dependent and was observed even at a low calcitriol concentration (10^{-10} M). Previous reports have shown that calcitriol reduces parathyroid cell proliferation (6) by decreasing proto-oncogene c-myc expression (7). This gene is involved in the regulation of the cell cycle (by modulating the progression of cells from the G1 into the S phase) and may also promote apoptosis in activated cells when it is anomalously expressed (28). Thus, the deficiency of calcitriol in parathyroid tissue cultures may cause both the progression into the cell cycle and the induction of apoptosis via a disinhibition of c-myc expression. The addition of calcitriol to the culture would have the opposite effect. Thus, it is not surprising to observe that the decrease in parathyroid cell proliferation induced by calcitriol is accompanied by a reduction in apoptosis.

The analysis of the freshly obtained parathyroid tissue from parathyroidectomized patients showed that adenomas had a higher proliferative activity than did secondary hyperplasia. Between the two forms of secondary hyperplasia, nodular hyperplasia had slightly higher proliferative rates than did diffuse hyperplasia. These findings are similar to those previously reported by others (30–32). In adenomatous and hyperplastic human parathyroid tissue, the number of apoptotic cells was low and was not significantly different between 1°HPT and 2°HPT. We cannot be certain that the percentage of apoptotic cells in hyperplastic parathyroid tissue was increased as compared with normal parathyroid tissue because normal parathyroid glands were not available for study. However, a previous publication reported that the percentage of apoptotic cells was greater in glands from patients with 2°HPT than in normal parathyroid tissue (25). In the literature, non-uniform data have been reported about the rates of apoptosis in 1°HPT and 2°HPT. Whereas some authors reported values as high as 5.3% and 9.5% of apoptotic cells for 1°HPT and 2°HPT, respectively (25), others have observed 0.35% and 0.1%, respectively (33); the latter values are close to our results. These differences may be due to the different methods used to quantify apoptosis.

The in vitro response of parathyroid tissues from secondary
hyperplasias to calcitriol was poor as compared with normal parathyroid tissue from dogs. Except at the highest concentration (10⁻⁷ M), calcitriol failed to inhibit cell proliferation and apoptosis. In adenomatous glands from patients with 1°HPT, no effect of calcitriol was observed; this may be interpreted as an even further degree of autonomy. In previous reports in uremic patients and rats with renal failure, parathyroid gland size was reported to decrease after calcitriol treatment and the proposed mechanism was a reduction in the number of cells as a result of an increase in cell apoptosis (20,34). However, other authors have not been able to demonstrate a decrease in parathyroid gland size in patients with 2°HPT treated with calcitriol (10,18,19). If the effect of calcitriol on the cell cycle and apoptosis that we have observed in the in vitro setting is similar to that in vivo, then the administration of calcitriol should not directly reduce the size of the parathyroid glands. The decrease in parathyroid cell proliferation induced by calcitriol might stop parathyroid gland growth. However, to reduce the mass of parathyroid tissue, it would be necessary also to increase the rate of apoptosis; the results of our in vitro experiments suggest that calcitriol at concentrations similar to those observed in vivo failed to induce apoptosis of parathyroid cells. Because of the coupling between proliferation and apoptosis, it seems unlikely, on the basis of our in vitro findings, that calcitriol changes the balance between the two processes. Moreover, it is commonly observed that in a number of renal transplant patients in whom calcitriol levels return to normal, parathyroid hyperplasia does not regress and eventually parathyroidec- tomy is required. This clinical observation suggests that in patients with advanced 2°HPT, the normalization of serum calcitriol levels after transplantation is not followed by a significant increase in parathyroid cell apoptosis. However, it must be stressed that the parathyroid glands used in this study were obtained from patients with severe 2°HPT who did not respond to calcitriol in vivo. Thus, regardless of the histologic nature of the hyperplasia, it is also possible that parathyroid glands from patients with earlier stages of 2°HPT, who respond to calcitriol in vivo, would be more sensitive to calcitriol in vitro.

Because parathyroid glands with nodular hyperplasias have been reported to have a greater decrease in VDR and to be more autonomous in function and growth than parathyroid glands with diffuse hyperplasia (15), we compared the effect of calcitriol on the cell cycle and apoptosis in nodular versus diffuse hyperplasia. Our results showed that there were no differences between the in vitro response in both types of hyperplasia. In earlier stages of parathyroid hyperplasia, cells from diffuse hyperplasia might be more susceptible to modulation by calcitriol (6). However, the present study was performed in glands from parathyroidec- tomized patients with advanced 2°HPT. Other authors (17) have detected monoclonal growth in glands with diffuse hyperplasia from parathyroidec- tomized patients with advanced 2°HPT, and it is conceivable that virtually all glands from patients with uncontrolled 2°HPT could be considered as nodular hyperplasias. A recent report showed a high proliferative capacity in parathyroid cells auto- transplanted to the forearm whether the graft included areas of nodular or diffuse hyperplasia (32).

In conclusion, we have shown in an in vitro model that calcitriol inhibited both proliferation and apoptosis of parathyroid cells from normal dogs. However, in contrast to the normal dog, the response to calcitriol in human parathyroid glands from patients with 1°HPT and those with advanced 2°HPT was marginal. The failure to respond in vitro is similar to the behavior of these parathyroid glands in vivo. Because of coupling between proliferation and apoptosis, it seems unlikely, on the basis of our in vitro findings, that calcitriol affects the balance between the two processes. Thus, a reduction of the parathyroid gland mass should not be expected as a direct effect of calcitriol treatment in patients with severe 2°HPT.

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