High Phosphate Level Directly Stimulates Parathyroid Hormone Secretion and Synthesis by Human Parathyroid Tissue In Vitro

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Abstract. Phosphate retention plays an important role in the pathogenesis of secondary hyperparathyroidism in patients with renal failure. In in vitro studies, high extracellular phosphate levels directly stimulate PTH secretion in rat and bovine parathyroid tissue. The present study evaluates the effect of high phosphate levels on the secretion of PTH and the production of prepro PTH mRNA in human hyperplastic parathyroid glands. The study includes parathyroid glands obtained from patients with primary adenomas and from hemodialysis and kidney-transplant patients with diffuse and nodular secondary hyperplasia. The experiments were performed in vitro using small pieces of parathyroid tissue. The ability of high calcium levels to decrease PTH secretion was less in adenomas than in secondary hyperplasia; among the secondary hyperplasia, nodular was less responsive to an increase in calcium than diffuse hyperplasia. In diffuse hyperplasia, PTH secretion was increased in response to 3 and 4 mM phosphate compared with 2 mM phosphate, despite a high calcium concentration in the medium; prepro PTH mRNA levels increased after incubation in 4 mM phosphate. Similar results were obtained with nodular hyperplasia, except that the elevation of PTH secretion in response to 3 mM phosphate did not attain statistical significance. In adenomas, high calcium concentrations (1.5 mM) did not result in inhibition of PTH secretion, independent of the phosphate concentration, and the prepro PTH mRNA was not significantly increased by high phosphate levels. In conclusion, first, the PTH secretory response to an increase in calcium concentration is less in nodular than diffuse hyperplasia; second, high phosphate levels directly affect PTH secretion and gene expression in patients with advanced secondary hyperparathyroidism.

Patients with chronic renal failure develop secondary hyperparathyroidism (1). Hypocalcemia, low calcitriol levels, and phosphorus retention are the key factors in the pathogenesis of secondary hyperparathyroidism (2–4).

Recent studies of animals and patients have confirmed the beneficial effect of dietary phosphorus restriction in the control of secondary hyperparathyroidism (5–8). In renal failure, phosphorus retention inhibits calcitriol production (9,10) and decreases the calcemic response to PTH (11,12), two mechanisms by which phosphorus retention promotes secondary hyperparathyroidism. Studies of rats have shown that low phosphate levels decrease (13) and high phosphate levels increase PTH secretion and gene expression (14), and recent in vitro studies demonstrate that high phosphate levels stimulate PTH secretion (15–17). All these reports indicate that high phosphate levels have a direct effect on parathyroid function.

An increase in the serum phosphate level is observed in patients with advanced renal failure when parathyroid hyperplasia is established. In these patients, parathyroid hyperplasia is often nodular with evidence of monoclonal growth (18). This nodular hyperplastic tissue has been shown to have a low density of calcitriol receptors (19) and decreased expression of calcium receptor mRNA and protein (20,21). It is not known whether these abnormal parathyroid cells respond similarly to phosphate as do normal rat and bovine parathyroid glands.

The aim of the present study was to investigate the effect of phosphate levels on PTH secretion and gene expression in parathyroid gland tissue from patients with primary and secondary hyperparathyroidism. Our results show that high phosphate levels stimulate PTH secretion and gene expression by human parathyroid tissue; in addition, we have observed that the PTH secretory response to an increase in calcium levels is less in nodular than diffuse hyperplasia.

Materials and Methods

The experiments were performed in human parathyroid tissue obtained from patients with parathyroid adenoma and hemodialysis and renal transplant patients with secondary hyperparathyroidism in whom parathyroidectomy was indicated. Patients with secondary hy-
periparathyroidism included 8 patients on chronic hemodialysis and 11 renal transplant patients with a functional transplant for more than 1 yr. All 10 patients with primary hyperparathyroidism had autonomic. The age of the patients ranged from 48 to 70 yr for those with primary hyperparathyroidism and from 25 to 66 yr for those with secondary hyperparathyroidism.

At the time of parathyroidectomy, serum PTH and total calcium were, respectively, 123 ± 13 pg/ml and 2.90 ± 0.09 mM for patients with primary hyperparathyroidism and 890 ± 122 pg/ml and 2.92 ± 0.05 mM for patients with secondary hyperparathyroidism. Hemodialysis and transplant patients had similar PTH levels (846 ± 192 versus 926 ± 166 pg/ml), and the mean total serum calcium concentration was elevated in both groups of patients, although higher for transplant than hemodialysis patients (3.04 ± 0.07 versus 2.80 ± 0.05 mM, P < 0.05). The mean serum phosphate concentration was 1.82 ± 0.11 mM for hemodialysis patients, 0.91 ± 0.15 mM for renal transplant patients, and 0.98 ± 0.10 mM for patients with primary hyperparathyroidism. All dialysis patients required maintenance hemodialysis, and the serum creatinine level was lower than 3 mg/dl in all renal transplant patients.

**Parathyroid Glands**

Immediately after parathyroidectomy, a sample of parathyroid gland tissue was separated and maintained at 4°C in RPMI medium with a calcium concentration of 1.5 mM for 16 to 18 h until the experimental test was performed. Before the initiation of experiments, the available tissue was divided into two fragments, one for in vitro tests of PTH secretion and gene expression and the other for routine microscopic examination. The piece of tissue used for histologic evaluation was immediately adjacent to the piece of tissue used in the in vitro experiments; therefore, the histologic findings were considered to be representative of the tissue used for in vitro testing. Nodular hyperplasia was observed in 46% of glands from hemodialysis patients and in 40% of glands from renal transplant patients.

**Incubation Media**

Parathyroid tissue was cut in small pieces of approximately 1 mm³ and placed resting inside a nylon basket in individual wells with 2 ml of incubation medium. The tissue was maintained in constant motion with gentle rocking (AOS-0; SBS Instruments SA, Badalona, Spain) at 37°C. The incubation medium was buffered, pH 7.4, and the solution contained (in mM) NaCl 125, KCl 5.9, MgCl₂ 0.5, CaCl₂ ranging from 0.4 to 1.5, Na-pyruvate 1, glutamine 4, glucose 12, Hepes 25 with insulin 0.1 IU/ml, bovine serum albumin 0.1%, penicillin G 100 IU/ml, and streptomycin 100 µg/ml. Phosphate was added in the form of NaH₂PO₄ and Na₂HPO₄ in 1:2 proportion to achieve final phosphate concentrations of 1, 3, and 4 mM. The parathyroid tissue was allowed to rest for 5 h in 1.25 mM calcium and a 1, 3, or 4 mM phosphate concentration, depending on each subsequent test. The ionized calcium concentration presented are the measured values using a selective electrode (model 634, Ciba Corning, Essex, England). All chemical products were obtained from Sigma (St. Louis, MO).

**Effect of Phosphate Level on PTH Secretion**

Preliminary experiments were performed to determine the feasibility of the in vitro model and the effect of the phosphate levels on PTH secretion. To test in vitro the secretory response to low calcium levels, the parathyroid tissue previously incubated in 1 mM P was exposed to two consecutive 1-h periods of 1.25 and 0.6 mM Ca. In a different experiment, which was designed to evaluate the effect of phosphate levels on PTH secretion; two equal tissue samples from the same gland were incubated for 5 h with phosphate concentrations of either 1 or 4 mM and the same 1.25 mM Ca concentration; then, the incubation medium was renewed, and during one last additional hour, the medium was collected for measurement of PTH. Because higher phosphate concentration could chelate calcium, an additional amount of CaCl₂ was added to the incubation media to achieve the desired calcium concentration (15,16). The target pH and calcium concentration were confirmed by direct measurement, using selective electrodes. The measured pH and ionized calcium in the media did not change after the 1-h incubation. The concentration of intact PTH in the incubation medium was measured using a human intact PTH IRMA kit (Nichols Institute, San Juan Capistrano, CA) with an intra-assay and interassay coefficient of variation of 4.3 and 4.7%, respectively.

Cell viability was assessed in mechanically dispersed cells; two 1-mm³ pieces of tissue were placed in a plastic well in a small volume (100 µl) of incubation medium, the tissue was carefully stripped apart using thin tweezers (Dumont #5) under a ×30 magnification dissecting microscope. This procedure was followed by gentle pipetting. Cells were exposed to two fluorochrome dyes, the green fluorodiacetate (Sigma) (15 min 37°C in the dark), which stains living cells, and to the red propidium iodide (Sigma) (5 min in the dark), which marks dead cells. Cells then were introduced into a flow cytometer (FACSscan, Becton-Dickinson) and were analyzed with the software LYSIS II (Becton-Dickinson). Only samples of parathyroid glands with greater than 90% of viable cells were included in the study. Incubation of tissue in medium with a high phosphate concentration did not affect parathyroid cell viability.

**Effect of Phosphate Level on the Inhibition of PTH Secretion by Calcium**

The parathyroid tissue fragments from each single gland (at least 20 mg) were incubated in 1 mM (control) and 3 mM phosphate, respectively; other tissue samples from other single glands were incubated in the control and 4 mM phosphate. The calcium concentration in the medium was 1.25 mM. After 5 h, the parathyroid glands were sequentially transferred, at hourly intervals, to other wells containing fresh solutions with the same concentration of phosphate and varying concentrations of calcium, i.e., 0.4, 0.6, 0.8, 1.0, 1.25, and 1.35 or 1.5 mM. According to recent studies (20,21) in parathyroid hyperplastic tissue from uremic patients, the amount of calcium receptor mRNA and protein is decreased in nodular compared with diffuse hyperplasia, and both were higher than in primary hyperparathyroidism. These results suggest that the regulation of PTH secretion by calcium in vitro may be different according to the type of hyperplasia. Thus, the secondary hyperplasia was classified as nodular if there was evidence of nodules, or diffuse if no nodules were observed. It is important to note that the tissue sample analyzed by microscopy was a part of the parathyroid sample used to test the in vitro response to calcium. After each experiment, DNA was measured by a modified diphenyl-amine method (22). The cell viability measured after completion of the experiment was greater than 70% in medium both with high and with normal phosphate levels.

**Effect of Phosphate Level on Prepro PTHm RNA**

Part of the parathyroid tissue was exposed to 1, 3, and 4 mM phosphate with a measured ionized calcium concentration of 1.25 mM during a period of 11 h with hourly replacement of fresh medium. The tissue sample was processed for the quantitation of prepro PTH mRNA as follows.
Probe. A human prepro PTH cDNA probe was obtained by RT-PCR. Briefly, a cDNA synthesis was carried out using 5 μg of total RNA from human parathyroid tissue following standard methods (23). A 390-bp cDNA fragment was obtained by PCR using the sense primer hPTH 5′ TACAGCTTAGCATAACCTGG 3′ and the anti-sense primer hPTH2 5′ CAGTTATCATCGCATGATGAGG 3′ (24). Amplification reaction was carried out using standard conditions as previously described (14). The PCR product was cloned in pCRII vector (Invitrogen Corp., San Diego, CA), and the insert specificity was confirmed sequencing (Sequenase system, Amersham, Arlington Heights, IL). By using plasmid DNA as template, a PCR reaction was carried out to amplify the specific insert that was used as probe, and the PCR product was purified with Sephadex G-50 (Pharmacia Biotech, Uppsala, Sweden) (25). About 25 ng of DNA were labeled by random priming using the Prime-a-Gene kit (Promega Corp., Madison, WI) and 32P-dCTP 300Ci/mmol (Amersharn, Braunschweig, Germany).

Northern Analysis. Total RNA was isolated from the parathyroid tissue samples using an acid-guanidine isothiocyanate-phenol-chloroform method (26). The yield was determined by spectrophotometry (25). About 15 μg of total RNA were subjected to denaturing electrophoresis fractionation, and the gels were photographed with 665 type Polaroid film. The resolved RNA was transferred to Hybond-N nylon membranes (Amersharn, Germany) by capillary blotting and was cross-linked to the membranes by ultraviolet light, as previously described (14). The membranes were prehybridized, hybridized with labeled probe, and washed as described elsewhere (27). Then the membranes were exposed to Cronex x-ray film (Dupont, DE) for 16 h. To quantitate the mRNA levels in each blot, x-ray films were subjected to densitometry of the 28S ribosomal RNA band from the Polaroid negative film was used to normalize mRNA levels.

Figure 1. Stimulation of PTH secretion by low calcium concentrations in human diffuse hyperplastic parathyroid tissue in vitro. Bars represent mean ± SEM; each value was obtained from duplicate experiments.

Figure 2. Stimulation of PTH secretion by high phosphate concentrations in human diffuse hyperplastic parathyroid tissue in vitro. Bars represent mean ± SEM; each value was obtained from duplicate experiments.

Statistical Analyses

Differences between more than two means were evaluated by ANOVA followed by the Duncan test. Paired or unpaired t test was used to compare two means obtained from experiments in the same or different samples of tissue. The results are expressed as the mean ± SEM.

Results

In parathyroid tissue with diffuse hyperplasia, a decrease in the calcium concentration from 1.25 to 0.6 mM produced an increase in the rate of PTH secretion (from 101 ± 32 to 332 ± 67 pg/μg DNA per h; P < 0.05) (Figure 1). Although the degree of increase in PTH secretion induced by low calcium levels was variable, all five parathyroid glands tested increased PTH secretion with the low calcium levels. The effect of high phosphate levels on PTH secretion is presented in Figure 2; the rate of PTH secretion by diffuse hyperplastic tissue incubated in 4 mM phosphate and 1.25 mM calcium was 458 ± 170 pg/μg DNA per h which was significantly greater than 110 ± 28 pg/μg DNA per h, (P < 0.05) observed in the presence of 1 mM P. All glands increased PTH secretion in 4 mM P.

There was a marked difference in the degree of inhibition of PTH secretion by calcium in nodular hyperplasia, diffuse hyperplasia, and adenomas. Nodular hyperplasia was less responsive to an increase in calcium than diffuse hyperplasia, and both types of secondary hyperplasia were more responsive to calcium than adenomas (Figure 3). A significant inhibition of PTH secretion in the adenoma tissue was observed only with a concentration of calcium as high as 1.5 mM. In nodular hyperplasia, a decrease in PTH secretion was observed with a
calcium concentration of 1 mM; however, higher calcium concentrations did not further reduce PTH secretion; thus, a calcium concentration as high as 1.5 mM decreased PTH secretion only to 65 ± 12% of maximal. Finally, in diffuse hyperplastic tissue, the PTH was significantly decreased with a calcium concentration of 0.8 mM calcium, and it was further reduced by increasing the calcium concentration; thus, in 1.5 mM calcium, the PTH secretion was 32 ± 4% of maximal.

The incubation of diffuse hyperplastic tissue in the in vitro PTH-calcium relationship (Figure 4a). With a calcium concentration ranging from 0.8 to 1.5 mM, the PTH secretion was greater in 3 than 1 mM phosphate. In the presence of 3 mM phosphate, a significant inhibition of PTH secretion was first observed with a calcium concentration of 1 mM, whereas in 1 mM phosphate a significant suppression of PTH secretion was obtained with 0.8 mM calcium; with 1.5 mM calcium, the respective PTH secretion in 1 and 3 mM phosphate was 32.4 ± 4 and 69.9 ± 7, respectively (P < 0.001 by unpaired t test). With 4 mM phosphate in the incubation medium, PTH secretion was not inhibited by a high calcium concentration and the PTH secretion was even higher with 1 mM than 0.6 mM calcium (P = 0.06). The PTH mRNA in hyperparathyroid tissue incubated in 4 mM phosphate was increased fourfold (P < 0.01, n = 5) with respect to its control, which consisted of an adjacent piece of the same gland incubated in 1 mM phosphate (Figure 5). In 3 mM phosphate, the PTH mRNA was not significantly increased with respect to its control (103 ± 8%, n = 11).

In parathyroid tissue with nodular hyperplasia, the degree of inhibition of PTH secretion was less with than with 1 mM phosphate; however, this difference did not reach statistical significance (Figure 4b). Similarly to the observation in diffuse hyperplasia, incubation with 4 mM phosphate prevented the inhibition of PTH secretion by calcium. Thus, in the range of calcium concentration from 0.4 to 1.5 mM, the PTH secretion did not change significantly. The PTH mRNA in 4 mM P was greater (P < 0.05, n = 5) than its control incubated in 1 mM phosphate (Figure 6).

A separate analysis was performed using parathyroid glands from renal transplant patients with either diffuse or nodular hyperplasia. We found no significant correlation between the PTH response to calcium and the serum creatinine. Similarly, the increase in the PTH secretion induced by 4 mM phosphate did not correlate with serum creatinine. It is important to note that most patients had only a very moderate decrease in renal function (serum creatinine < 2 mg/dl).

In parathyroid adenoma, PTH secretion was not inhibited by the increase in calcium concentration independent of the concentration of phosphate in the incubation medium (Figure 4c). In 4 mM phosphate, the PTH mRNA level was greater than its control, but statistical significance was not present because only three glands were examined (Figure 5). In 3 mM phosphate, the PTH mRNA level was similar as its control (97 ± 11%, n = 4).

**Discussion**

The present study shows that, in vitro, a high phosphate concentration increases PTH secretion and PTH mRNA production in human hyperplastic parathyroid tissue. The effect of phosphate was evaluated separately in nodular and diffuse secondary hyperplasia, and in parathyroid adenomas. High calcium levels produced a greater inhibition of PTH secretion in secondary hyperplasia than in adenomas; among the secondary hyperplasias, nodular hyperplasia was less sensitive to changes in calcium concentration than was the diffuse hyperplasia. The high phosphate levels prevented the inhibition of PTH secretion by calcium, and this finding was more evident in diffuse than nodular hyperplasia because inhibition was greater with diffuse hyperplasia. In adenomas, the inhibition of PTH secretion by high calcium levels was marginal, and high phosphate levels did not significantly affect this abnormal PTH response to calcium.

Experiments were performed using small pieces of parathyroid glands instead of dispersed parathyroid cells. In this in vitro system, low and high calcium concentration produce stimulation and inhibition of PTH secretion respectively; therefore, this model is suited to test the regulation of PTH secretion in vitro. This same model of rat and bovine parathyroid tissue culture has been previously used by us and other investigators to demonstrate a direct effect of phosphate levels on PTH secretion (15–17). Our results show that in human hyperplastic

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**Figure 3.** The inhibition of PTH secretion by calcium "in vitro" in human parathyroid adenoma (squares, HPTH-1°, n = 10), nodular hyperplasia from hemodialysis and renal transplant patients with secondary hyperparathyroidism (filled circles, HPTH-2° nod., n = 15), and diffuse hyperplasia also from hemodialysis and renal transplant patients with secondary hyperparathyroidism (open circles, HPTH-2° dif., n = 21). Values of PTH are the percentages (mean ± SEM) of maximal stimulation. Values of maximal PTH stimulation for HPTH-1°, HPTH-2° nod., and HPTH-2° dif. were 292 ± 75, 371 ± 95, 423 ± 73 pg of PTH/μg DNA per h, respectively. *P < 0.05 versus HPTH-1°, and *P < 0.05 versus HPTH-2° dif. From calcium concentrations of 0.8 to 1.5 mM, the reduction of PTH was significantly greater (P < 0.01) in HPTH-2° dif. than HPTH-1°.
parathyroid tissue, similar to other species, a high phosphate concentration in the incubation medium increases PTH secretion, despite no change in the concentration of extracellular calcium. The concentration of ionized calcium in the medium was carefully monitored because a high phosphate concentration produces a fall in ionized calcium. Additional calcium was added to the medium to compensate for the excess of phosphate, and the concentrations of phosphate and calcium were pg PTH/μg DNA per h for 1, 3, and 4 mM phosphate, respectively. Pound signs and asterisks represent $P < 0.05$ versus 1 mM and 4 mM phosphate, respectively. From calcium concentrations of 0.8 to 1.5 mM, the reduction in PTH secretion was significantly greater in 1 than 4 mM phosphate ($P < 0.01$). (B) The inhibition of PTH secretion by calcium in nodular hyperplasia from hemodialysis and renal transplant patients with secondary hyperparathyroidism. The parathyroid tissue was incubated with phosphate concentrations of 1 mM (open circles, $n = 21$), 3 mM (filled circles, $n = 18$), and 4 mM (squares, $n = 5$). Values of PTH are the percentages (mean ± SEM) of maximal stimulation (371 ± 95, 410 ± 121, and 403 ± 137 pg PTH/μg DNA per h for 1, 3, and 4 mM phosphate, respectively). Asterisks represent $P < 0.05$ versus 3 and 4 mM phosphate. From calcium concentrations of 0.8 to 1.5 mM, the PTH reduction was not significantly different in 3 and 4 mM phosphate. (C) The secretion of PTH at different calcium concentrations in parathyroid adenoma from patients with primary hyperparathyroidism. The parathyroid tissue was incubated with phosphate concentrations of 1 mM (open circles, $n = 10$), 3 mM (filled circles, $n = 6$), and 4 mM (squares, $n = 6$). Values of PTH are the percentages (mean ± SEM) of the values obtained at 0.6 mM calcium (292 ± 75, 487 ± 136, and 512 ± 182 pg PTH/μg DNA per h for 1, 3, and 4 mM phosphate, respectively). Increasing calcium concentrations up to 1.5 mM did not reduce the PTH secretion at any of the phosphate concentrations tested.
carefully maintained at 4 and 1.5 mM, respectively. The importance of using tissue slices rather than dispersed cells to evaluate the effect of phosphate levels on PTH secretion was demonstrated by Nielsen et al. (17). In that study, the effect of phosphate levels on PTH secretion was evaluated using both tissue slices and dispersed bovine parathyroid cells; stimulation of PTH secretion by high extracellular phosphate levels was observed only in parathyroid slices. By contrast, in dispersed parathyroid cells, although PTH secretion changed appropriately in response to variations in extracellular calcium levels, high extracellular phosphate levels did not change PTH secretion significantly. The mechanism by which the parathyroid cell senses the change in extracellular phosphate levels is unknown; if a cell membrane protein mediates the effect of phosphate, it may be affected by tissue digestion, which is required for cell dispersion. However, that the effect of phosphate on PTH secretion is observed only after 4 to 5 h of incubation does not suggest the presence of a cell membrane receptor for phosphate. Other authors have shown that the expression of the calcium sensor mRNA decreases rapidly in dispersed parathyroid cells in culture (28,29); this may affect calcium-regulated PTH secretion in vitro. It is possible that intercellular communication is required to demonstrate the effect of phosphate levels on PTH secretion and gene expression. Arachidonic acid is involved in the inhibition of PTH secretion by calcium (30). We have shown that high phosphate levels affect the production of arachidonic acid by parathyroid cells (31); it is possible that, as in other systems (32), eicosanoids products produced by a cell may affect neighboring cells. This influence is a possible reason whereby intact parathyroid tissue architecture may be required to see an effect of phosphate.

An effect of phosphate levels on PTH secretion and PTH mRNA production has been demonstrated in normal rat and bovine parathyroid tissue (14–17). The main objective of this study was to evaluate the effect of phosphate levels on PTH secretion in hyperplastic human parathyroid tissue. This is important for two reasons: first, hyperphosphatemia is usually present in patients with end-stage renal disease requiring hemodialysis, and such patients usually have advanced secondary parathyroid hyperplasia; and second, these parathyroid glands differ from normal inasmuch as they frequently have areas of nodular growth formed by cells that have undergone genetic transformations (18) and possess a decreased number of vitamin D and calcium sensor receptors (19–21). In addition, the PTH response to calcium is abnormal in primary (33,34) and secondary hyperparathyroidism (35–37); in hyperplastic parathyroid tissue, the concentration of calcium required to inhibit PTH secretion is greater than normal (35–37).

To evaluate the effect of high phosphate levels on PTH secretion in an abnormal calcium-regulated parathyroid tissue, we considered that it was important to analyze PTH secretion throughout a wide range of calcium concentrations to construct the PTH-calcium curve. We found that in parathyroid adenomas PTH secretion was less responsive to an increase in extracellular calcium than in secondary hyperplasia; between secondary hyperplasias, nodular was less responsive than diffuse. Other authors have found loss of heterozygosity encompassing the calcium sensor receptor locus in approximately 10% of sporadic parathyroid adenomas (38), and it is possible that in parathyroid adenomas calcium sensor receptor biosynthesis is modulated through an alternative RNA processing (39). These data are consistent with our results in vitro showing a decreased calcium sensitivity in parathyroid adenomas. The decreased response to calcium observed in nodular hyperplasia may be explained by a reduced expression of calcium sensor receptor as recently demonstrated by Gogushev (21). The presence of nodules in the hyperplastic gland may be responsible for the increased setpoint observed in patients with severe secondary hyperparathyroidism (40).

In diffuse hyperplastic tissue, a high concentration of phosphate in the incubation media prevented the calcium-induced inhibition of PTH secretion. This effect was more marked with 4 than 3 mM P, suggesting a dose-response effect. The stimulation of PTH secretion by high phosphate levels (4 mM) was accompanied by an increase in prepro PTH mRNA; however, 3 mM phosphate did not increase PTH mRNA in vitro. It is possible that a longer period of incubation may have resulted in

![Figure 6. Quantification of prepro PTH mRNA by Northern blot. Parathyroid tissue with diffuse hyperplasia was incubated during 11 h in 1 mM P (P1) and 4 mM P (P4) with 1.25 mM Ca.](image-url)
PTH mRNA increase or that simply concentrations of phosphate higher than 3 mM are required to produce an increase in PTH mRNA. Data on PTH mRNA is available only after 11 h of incubation; therefore, the present experiments do not provide information about the time required to observe an effect of phosphate on PTH mRNA production. In nodular hyperplasia, the inhibition of PTH secretion by calcium was impaired even with a normal phosphate concentration. Similar to that observed with diffuse hyperplasia, high phosphate levels in the medium further decreased the ability of high calcium to inhibit the PTH secretion; however, the reduction of PTH secretion by calcium was not significantly different between 3 and 1 mM phosphate. High phosphate concentration (4 mM) also increased the production of prepro PTH mRNA in nodular hyperplasia.

In parathyroid adenoma, high phosphate levels in the medium did not affect the already disturbed regulation of PTH by calcium; it is possible that in adenomas cells are relatively unresponsive to any of the regulatory mechanisms, including phosphate level. The observation of a less parathyroid cell response to phosphate levels in adenomas than nodular hyperplasia and less response in nodular than diffuse hyperplasia suggest that the parathyroid cells are equipped with physiologic mechanisms to respond to extracellular phosphate, and these mechanisms are affected by an abnormal growth.

Our results explain why, in uremic patients with a high serum phosphate level, calcitriol administration fails to control hyperparathyroidism (41). The concentration of phosphate used in these in vitro experiments are relatively high compared with the 1.25 to 2.5 mM phosphate concentrations usually seen in dialysis patients; apart from the obvious differences between in vitro and in vivo situations, it should be considered that the in vitro experiment is acute, whereas in vivo, the elevated levels of phosphate are maintained. Furthermore, the concentration of phosphate required to produce an effect on PTH secretion may be less in vivo than in vitro; Hernandez et al. (14) have shown that in rats, small changes in serum phosphate levels induced by a high phosphorus diet resulted in a significant increase in PTH secretion and gene expression. From the results obtained in the present study it is clear that medical treatment of secondary hyperparathyroidism requires a strict control of serum phosphate levels, not only because high phosphate levels decrease the calcemic effect of PTH, but also because high phosphate levels stimulate parathyroid cells directly.

All these results demonstrate that a high phosphate level stimulates PTH secretion and gene expression by human parathyroid tissue. This effect is independent of a low calcium concentration and a calcitriol deficiency, which are usually present in uremic patients; thus, the control of phosphate levels in patients with renal failure is essential for the prevention and control of secondary hyperparathyroidism. In addition, we have observed that the PTH secretory response to an increase in calcium is less in nodular than diffuse hyperplasia, which may explain the abnormal PTH regulation by calcium in severe secondary hyperparathyroidism.

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