Activation of the Calcium Receptor by a Calcimimetic Compound Halts the Progression of Secondary Hyperparathyroidism in Uremic Rats

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Abstract. The secondary hyperparathyroidism that develops in rats with chronic renal insufficiency (CRI) can be totally prevented by activation of the parathyroid Ca\(^{2+}\) receptor with a calcimimetic compound, when treatment is initiated before parathyroid cell hyperplasia and increased circulating parathyroid hormone levels develop. In clinical practice, however, secondary hyperparathyroidism is usually manifest by the time CRI is diagnosed. This study examined the effects of daily oral gavage or continuous subcutaneous infusion for 8 wk of the calcimimetic NPS R-568 on the progression of established mild or moderate-to-severe secondary hyperparathyroidism in rats with CRI induced by 5/6 nephrectomy. Both oral and infused NPS R-568 completely prevented further hyperplasia but did not reduce total parathyroid cell number below that present at the initiation of treatment. This prevention of cellular proliferation occurred despite increases in plasma phosphate and decreases in Ca\(^{2+}\) and 1,25-dihydroxyvitamin D levels, and supports the view that the Ca\(^{2+}\) receptor is the dominant regulator of parathyroid cell hyperplasia in addition to parathyroid hormone secretion. The clinical implications of these findings suggest that controlling Ca\(^{2+}\) receptor activity with calcimimetic compounds could be sufficient to manage secondary hyperparathyroidism in CRI.

Secondary hyperparathyroidism (2°HPT), characterized by enlargement of the parathyroid glands and increased levels of plasma parathyroid hormone (PTH), is a nearly invariant complication of end-stage renal disease. Parathyroid gland enlargement results mostly from cellular hyperplasia in all four parathyroid glands and is positively correlated with the magnitude of circulating levels of PTH (1–5). Increased levels of plasma PTH are often detected long before the start of dialysis when GFR has fallen to only half of normal. Because of this, it is likely that parathyroid cell hyperplasia also occurs early in the course of renal failure. Indeed, studies of chronic renal insufficiency (CRI) in rodents reveal increased proliferation of parathyroid cells within days of a partial nephrectomy (6,7). There is general agreement that lowered plasma levels of 1,25-dihydroxyvitamin D\(_3\) (1,25(OH)\(_2\)D\(_3\)), hypocalcemia, and hyperphosphatemia, as well as decreased expression in the parathyroid gland of the Ca\(^{2+}\) and 1,25(OH)\(_2\)D\(_3\) receptors, all contribute to parathyroid cell hyperplasia (7–17). However, the relative role of these systemic factors is controversial, and each has been postulated to play a dominant role in the pathogenesis of 2°HPT. This controversy results largely from the difficulty in manipulating the target mechanisms of these factors independently of the others. Thus, the mechanism by which phosphate regulates parathyroid cell proliferation is unknown, and changing 1,25(OH)\(_2\)D\(_3\) receptor activity is difficult to achieve in the absence of corresponding changes in plasma levels of phosphate and/or Ca\(^{2+}\). At present, the Ca\(^{2+}\) receptor is the only target mechanism that can be regulated independently of plasma levels of Ca\(^{2+}\), 1,25(OH)\(_2\)D\(_3\), or phosphate. This is possible because potent and selective pharmacologic activators (calcimimetics) of this receptor have been identified.

The use of calcimimetic compounds in rodent models of 2°HPT has provided evidence demonstrating an important role of the Ca\(^{2+}\) receptor in regulating not only secretion of PTH but additionally parathyroid cell proliferation. Thus, the very rapid proliferative response of parathyroid cells to a partial nephrectomy is prevented by the calcimimetic compound NPS R-568 when administered from the time of the renal insult (6). In long-term studies, daily dosing with NPS R-568 completely prevented the development of 2°HPT as indexed by parathyroid cell hyperplasia and circulating levels of PTH (18). Although these studies have contributed to the growing preclinical and clinical literature supporting the feasibility of this approach to treating hyperparathyroidism, they do not mimic the situation faced by the clinician. Thus, by the time the patient is diagnosed with CRI, which may lead to end-stage renal disease, 2°HPT is typically already present to some degree (5). In the foreseeable future, the treatment of 2°HPT...
will reflect managing an existing disease and should focus on treatments that will prevent it from progressing to more severe forms. It is this clinical reality that underlies the present experiments, which examined whether chronic treatment with NPS R-568, administered either by daily oral gavage or by continuous subcutaneous infusion, could reverse the hyperplasia and parathyroid gland enlargement in rats with CRI and established 2°HPT.

Materials and Methods

Animals, Diets, and Surgery

Normal male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), 7- to 8-wk-old and weighing 225 to 250 g upon receipt, were used in these studies. They were provided with unlimited access to commercial rodent chow (Purina no. 5001), containing 1% Ca and 0.7% P, and tap water. After an acclimatization period of 6 to 8 d, each rat was anesthetized with ketamine (90 mg/kg) and xylazine (7 mg/kg) injected intramuscularly and subjected to a one-stage 5/6 nephrectomy (Nx) in which both poles of one kidney were ligated and excised and the contralateral kidney was removed. Control rats were subjected to a sham operation, which involved exposure of both kidneys via two separate flank incisions and subsequent closure of the incisions. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee of NPS Pharmaceuticals, Inc. (Salt Lake City, UT).

Experimental Protocols

Experiment 1 (Mild 2°HPT). The rats were fed normal rodent chow throughout this study. Four weeks after surgery, the sham-operated and 5/6 Nx rats were divided into two and three weight-matched groups, respectively. One group of sham and one group of 5/6Nx rats were euthanized as baseline controls. The remaining group of sham-operated rats and the two groups of 5/6 Nx rats were anesthetized with isoflurane, and a model 2ML4 osmotic mini-pump (Alza, Palo Alto, CA) was implanted subcutaneously between the shoulder blades. The sham-operated rats and one group of 5/6 Nx rats were infused with the vehicle, a 45% aqueous solution of 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals International, Natick, MA); the remaining group of 5/6 Nx rats was infused with NPS R-568 (20 μmol/kg per d, subcutaneously). After 4 wk, the rats were anesthetized with isoflurane and new pumps were implanted to continue the infusions for an additional 4 wk. The concentration of NPS R-568 in the infusate was adjusted to account for the increase in body weight so that the infusion rate was maintained at about 20 μmol/kg per d. For euthanasia, the rats were anesthetized with ketamine and xylazine and exsanguinated by cardiac puncture into a heparinized syringe. Plasma Ca²⁺ levels were measured immediately, and the remaining plasma was stored at −20°C for subsequent analysis. Both parathyroid glands were separated from associated thyroid tissue by careful blunt dissection using a binocular dissecting microscope and processed as described below.

Experiment 2 (Moderate-to-Severe 2°HPT). The rats were allowed to recover from surgery for 3 d, during which time they were offered normal rodent chow. Then, all rats were transferred to a semi-synthetic diet that contained 0.6% Ca, 0.8% P, and 2.2 IU vitamin D₃/g (TD 95211; Harlan Teklad, Madison, WI). The P:Ca ratio of this diet is twofold higher than normal rodent chow and provides a greater drive to the development of 2°HPT. The rats were maintained on this diet and were weighed weekly throughout the remainder of the study. At 8 to 9 wk after surgery, each rat was anesthetized with isoflurane and a blood sample was collected from the tail for determination of the plasma levels of urea nitrogen, Ca²⁺, phosphate, and PTH.

Acute Effects of NPS R-568 on Plasma Levels of PTH and Ca²⁺. 5/6 Nx animals with moderate 2°HPT were catheterized and, 3 to 5 d later, the time course of the changes in the plasma levels of PTH and Ca²⁺ following a single oral administration (1 ml/200 g body wt) of NPS R-568 (10 or 30 μmol/kg) or vehicle (10% cyclodextrin in water) was assessed. Blood samples (0.6 ml) were collected immediately before, and at 15, 30, 60, 90, 120, 180, and 240 min after dosing. To prevent excessive blood loss, after removal of the plasma sample, the red cell pellet was resuspended in an equal volume of normal rat plasma and reinjected.

Effects of Chronic Treatment with NPS R-568. The sham-operated rats were divided into two groups (groups 1 and 3) and the remaining 5/6 Nx animals into five groups (groups 2, 4, 5, 6, and 7) matched by body weight and by the measured plasma parameters. Groups 1 and 2 were euthanized as untreated baseline controls at 11 wk after surgery. Groups 3 and 4 received vehicle (10% cyclodextrin) by oral gavage once daily for 8 wk in a volume of 1 ml/200 g body wt. Groups 5 and 6 received NPS R-568 orally (10 or 30 μmol/kg, respectively). Group 7 rats received NPS R-568 by subcutaneous infusion in a manner identical to that described above in Experiment 1. As before, the rats were anesthetized with isoflurane and the osmotic minipump was replaced at 4 wk. The animals were weighed weekly throughout the study and were euthanized for tissue collection as described above.

Parathyroid Gland Analysis

The parathyroid glands were fixed overnight in cold (4°C), neutral, phosphate-buffered formalin and dehydrated in ethanol. The glands were embedded in Epon (Tedpella, Tustin, CA) in Experiment 1, and in JB-4 resin (Electron Microscopy Sciences, Fort Washington, PA) in Experiment 2 to facilitate more rapid sectioning. Semithin (3 μm) sections of both glands of each rat were prepared and stained with basic fuschin and methylene blue and were examined by light microscopy using oil immersion and phase contrast techniques.

Total gland volume, average cell volume, and total cell number were determined using stereologic techniques (12,19,20). The volume of each gland (V₆) was estimated from parallel sections separated by a known distance t. The profile area (a[prof]) of each parallel section between interval sections was measured using a digitizer. Thus, V₆ = t × Σ(a[prof]). Total gland volume was determined by adding the volumes of the paired glands.

The volume densities (volume per unit tissue volume) of epithelial cells and the volume densities of epithelial cell nuclei were estimated by point counting as described previously (12,19). Eight test areas (100 μm × 100 μm) were selected by random systematic subsampling from four random sections of each parathyroid gland and were analyzed with a Nikon eyepiece containing 100 test points. The numerical density of epithelial cell nuclei were calculated assuming their shapes were spherical. Total parathyroid cell number (n) per rat was estimated from numerical density (Nᵥ) of cell nuclei in each gland multiplied by the volume of each gland: n = (Nᵥ₁ × V₆₁) + (Nᵥ₂ × V₆₂).

Plasma Analysis

Plasma Ca²⁺ levels were measured using a model 634 Ca²⁺ analyzer (Ciba Corning Diagnostics, Medford, MA). In Experiment 1, plasma urea nitrogen levels were measured using a kit (Sigma no. 640), plasma creatinine levels using a Technicon RA-500 autoana-
lyzer system, and plasma phosphate levels using the method of Chen et al. (21). In Experiment 2, these variables were measured using a multichannel analyzer (Monarch 1000; Instrumentation Laboratory, Lexington, MA). In both experiments, plasma PTH levels were measured using a rat PTH-(1-34) immunoradiometric assay (Immutopics, San Clemente, CA) and 1,25(OH)2D3 levels using a calf thymus radioreceptor assay (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra- and interassay coefficients of variation in the 1,25(OH)2D3 assay averaged 9.1 and 8.3%, respectively.

### Statistical Analyses

The parathyroid gland and plasma data presented are only from animals from which both glands were collected undamaged. The results shown are means ± the SEM and were initially subjected to ANOVA. When F ratios were significant, the Fisher protected least significant difference multiple comparison test was used to determine the significance of differences between groups. Correlations between serum parameters and parathyroid gland volume, and cell number and volume were analyzed using the Pearson correlation test (StatView, SAS Institute, Cary, NC).

### Results

#### Experiment 1 (Mild 2°HPT)

Plasma levels of creatinine, blood urea nitrogen (BUN), and PTH were elevated significantly by 2.0-, 3.3-, and 2.3-fold, respectively, confirming the induction of mild CRI and the development of a mild 2°HPT in the 5/6 Nx animals at baseline, 4 wk after the 5/6 Nx procedure. In contrast, there were no significant differences in the plasma levels of Ca2+ or phosphate at baseline. Plasma 1,25(OH)2D3 levels, however, were significantly lower in 5/6 Nx rats (Figure 1). There were no significant increases over baseline in the plasma levels of creatinine, BUN, or PTH in the vehicle-infused 5/6 Nx rats by the end of the study at 12 wk after surgery. This indicated that neither the magnitude of the CRI nor the 2°HPT had increased in control, vehicle-infused CRI rats during the 8-wk treatment period. There were also no significant changes in plasma levels of Ca2+ or phosphate in vehicle-infused sham-operated or 5/6 Nx rats during the treatment phase of the study. In contrast, 1,25(OH)2D3 levels decreased significantly from baseline in both groups (Figure 1).

The infusion of NPS R-568 for 8 wk in rats with CRI was not associated with any changes in urea nitrogen or creatinine levels, suggesting that this compound did not influence the impaired renal function in these animals. However, the elevated plasma PTH levels in vehicle-infused CRI rats was completely reversed by the infusion of NPS R-568, and the reduction in PTH levels was associated with a decrease in the plasma levels of Ca2+ (Figure 1). The reduction in plasma PTH levels by NPS R-568 in CRI rats was not associated with any significant changes in plasma phosphate or 1,25(OH)2D3 levels, although average 1,25(OH)2D3 levels were 52% lower (Figure 1).

Rats with CRI tended to weigh less than sham-operated controls at baseline, but body weight increased by about the same amount in both groups during the 8-wk treatment phase of the study. This body weight gain was reduced slightly in the CRI rats that received NPS R-568 such that they were significantly lighter than sham controls, but not CRI controls, at the end of the study (Figure 2). Total parathyroid gland volume increased in both sham and control CRI rats during the study in direct proportion to the increase in body weight. When normalized to body weight, gland volume was 84% larger in CRI rats with CRI.
rats than in sham controls at baseline, but only 92% larger at the end of the 8-wk treatment period, confirming the lack of significant progression of 2°HPT during the study. The increased gland volume in CRI rats was caused primarily (>80%) by cellular hyperplasia and secondarily by cellular hypertrophy (Figure 2). The infusion of NPS R-568 for 8 wk reduced total parathyroid gland volume to a level that was not significantly different from that in sham controls. This decrease in gland volume resulted exclusively from a decrease in average cell volume (Figure 2).

Experiment 2 (Moderate-to-Severe 2°HPT)

Acute Effects of NPS R-568 on Plasma Levels of PTH and Ca²⁺. The time course of changes in the plasma levels of PTH and Ca²⁺ after the oral administration of NPS R-568 at the doses used in the chronic study were evaluated in 5/6 Nx rats with moderate 2°HPT. Plasma PTH levels averaged 193 pg/ml in the rats with CRI, compared with 34 pg/ml in sham-operated rats fed the same high phosphate diet (not shown). PTH levels increased about twofold during the study in rats that received vehicle, a change that was associated with a decrease in plasma Ca²⁺ levels in that group (Figure 3). Both oral doses of NPS R-568 significantly decreased PTH levels within 15 min. Although the average decrease in PTH levels was greatest both in magnitude and duration with the 30 μmol/kg dose, there were no significant differences in PTH levels between doses at any time point. PTH levels remained significantly lower than those in vehicle-dosed rats for only 60 min, although the average PTH levels remained lower than in control animals throughout the study. The decrease in PTH levels resulted in a dose-dependent decrease in plasma Ca²⁺ levels. With the 10 μmol/kg dose, Ca²⁺ levels were signifi-

Figure 2. Effect of continuous subcutaneous infusion of NPS R-568 (20 μmol/kg per d) or vehicle for 8 wk starting 4 wk after surgery (baseline) on body weight and parathyroid gland morphology in sham-operated rats and in 5/6 nephrectomized rats with CRI. All rats were fed normal rodent chow throughout the study. Values are mean ± SEM, n = 4 per group. *a,bP < 0.05, significance of difference versus corresponding sham group or the CRI-vehicle group, respectively.

Figure 3. Effect of oral administration of NPS R-568 or vehicle on plasma levels of parathyroid hormone (PTH) and Ca²⁺ in 5/6 nephrectomized rats with moderate secondary hyperparathyroidism induced by feeding a 0.6% Ca, 0.8% P diet for 9 to 10 wk after surgery. Values are mean ± SEM, n = 5 to 6 per group. *P < 0.05 versus vehicle-dosed controls.
cantly lower from 2 to 3 h, whereas the rate of onset of significant hypocalcemia was more rapid (90 min), and its duration was longer (>4 h), in rats that received the 30 μmol/kg dose (Figure 3).

**Effects of Chronic Treatment with NPS R-568.** At baseline, 11 wk after the surgical induction of CRI, plasma levels of creatinine, BUN, and PTH were elevated 1.9-, 2.7-, and 5.4-fold, respectively, in 5/6 Nx rats fed the high phosphate diet (Figure 4). The magnitude of uremia did not increase further in vehicle-treated CRI rats during the 8-wk treatment period. In contrast, PTH levels increased an additional 2.6-fold (to 608 pg/ml) in these control animals. Plasma Ca\(^{2+}\) levels tended to be lower and phosphate levels higher in control CRI rats both at baseline and at the end of the study, but none of the differences was statistically significant. As seen in Experiment 1, 1,25(OH)\(_2\)D\(_3\) levels were significantly lower in control CRI rats at baseline, but not at the end of the study (Figure 4).

Neither orally administered nor infused NPS R-568 affected the degree of uremia in the CRI rats (Figure 4). At 24 h after the last oral dose, plasma PTH levels in rats receiving the 10 μmol/kg dose of NPS R-568 were the same as in those given vehicle. However, in rats receiving the 30 μmol/kg dose, plasma PTH remained at a level (182 pg/ml) equivalent to that observed in the CRI rats at baseline (235 pg/ml). Moreover, the subcutaneous infusion of NPS R-568 reduced PTH to a level (43 pg/ml) similar to that in sham-operated controls. There were no significant differences in the plasma levels of Ca\(^{2+}\) or phosphate when measured at 24 h after the last oral dose of NPS R-568. In contrast, the infusion of NPS R-568 was associated with a marked hypocalcemia and hyperphosphatemia. There was also a tendency for a dose-dependent decrease in the plasma levels of 1,25(OH)\(_2\)D\(_3\), but the decrease was significant only in the animals infused with NPS R-568 (Figure 4).

As was observed in Experiment 1, uremia *per se* had no significant effects on body weight. Infusion, but not oral administration of NPS R-568, reduced the gain in body weight slightly in the uremic rats (Figure 5). When normalized to body weight, total parathyroid gland volume was 1.9-fold larger in 5/6 Nx rats at baseline but 4.1-fold higher at the end of the study, showing the progression of 2°HPT in the CRI animals. As in Experiment 1, the increase in gland volume was primarily the result of cellular hyperplasia rather than hypertrophy. Cell number was increased 1.7- and 3.2-fold, and average cell volume increased 12 and 28% at baseline and at the end of the study, respectively. The oral administration of NPS R-568 was associated with a dose-dependent decrease in total gland volume, although the decrease was statistically significant only with the 30 μmol/kg dose. This decrease resulted primarily from reduced parathyroid cell number; the effects of oral NPS R-568 on average cell volume were inconsistent. The total number of parathyroid cells in the CRI rats that received the 10 μmol/kg dose of NPS R-568 was significantly higher at the end of the study than at baseline, whereas there was no significant increase in cell number in rats given the 30 μmol/kg dose. This prevention of the further increase in gland volume and cell number was even more pronounced in uremic rats that received NPS R-568 continuously by infusion. Both parathyroid gland volume and cell number remained at the level seen in CRI rats at baseline. Average cell volume was also reduced in the infused rats to a level that was not significantly different from the sham-operated rats (Figure 5).
Correlation Analyses

To evaluate the mechanisms that led to parathyroid gland enlargement in CRI, we performed correlation analysis of the factors believed to play a role in this growth, i.e., plasma levels of Ca\(^{2+}\), phosphate, and 1,25(OH)\(_2\)D\(_3\). This analysis included all results from rats in which we performed stereologic analysis of the parathyroid glands and measured these plasma parameters. To reduce variability, the plasma parameters were correlated against total parathyroid gland volume and cell number normalized to body weight; average cell volumes were not normalized. As expected, plasma PTH levels were strongly correlated with gland volume (Figure 6). However, the correlations of gland volume against plasma Ca\(^{2+}\) and phosphate levels, although statistically highly significant, were not as strong. There was no correlation between gland volume and 1,25(OH)\(_2\)D\(_3\) levels.

A more detailed analysis of the correlations between parathyroid gland volume, cell volume, and cell number with plasma levels of PTH, Ca\(^{2+}\), phosphate, and 1,25(OH)\(_2\)D\(_3\) was made in which the effect of treatment with NPS R-568 was analyzed separately (Table 1). In untreated animals (sham and CRI, baseline, and vehicle-treated), parathyroid gland volume was significantly correlated with serum PTH, Ca\(^{2+}\), and phos-

Figure 5. Effect of daily oral gavage (po) or continuous subcutaneous (sc) infusion of NPS R-568 or vehicle for 8 wk starting 11 wk after surgery (baseline) on body weight and parathyroid gland morphology in sham-operated rats and in 5/6 nephrectomized rats with CRI. All rats were fed a 0.6% Ca, 0.8% P diet throughout the study. Values are mean ± SEM, n = 4 to 5 per group. \(a^b\)P < 0.05, significance of difference versus corresponding sham group or the CRI-vehicle group, respectively.

Figure 6. Correlation of plasma levels of PTH, Ca\(^{2+}\), phosphate, and 1,25(OH)\(_2\)D\(_3\) against total parathyroid gland volume. Each point represents an individual sham-operated or 5/6 nephrectomized rat with established 2°HPT that was untreated (○) or received NPS R-568 (●).
Calcimimetic compounds such as NPS R-568 are potent and selective activators of the Ca\(^{2+}\) receptor in vitro and preferentially target the parathyroid cell Ca\(^{2+}\) receptor in vivo (22–25). This action of NPS R-568 completely explains the rapid fall of plasma PTH levels when the compound is administered to normal rodents or to those with 2\(^{\text{nd}}\)HPT resulting from CRI (24–27). This single action, which results in an inhibition of PTH secretion, also largely explains the hypocalcemic response to NPS R-568. These pharmacologic properties suggest that compounds related to NPS R-568 might be useful in treating both primary and 2\(^{\text{nd}}\)HPT. Indeed, pilot studies using NPS R-568 in patients with primary (28) or 2\(^{\text{nd}}\)HPT (29,30) have demonstrated the efficacy and safety of this compound in both forms of hyperparathyroidism.

Although plasma levels of Ca\(^{2+}\) have been implicated in controlling parathyroid cell growth and PTH synthesis (9,31), there is no evidence demonstrating that these effects of Ca\(^{2+}\) are mediated by the Ca\(^{2+}\) receptor. As noted in the introductory remarks, it is difficult to manipulate the plasma levels of Ca\(^{2+}\) independently of those of phosphate and 1,25(OH)\(_2\)D\(_3\). The use of calcimimetic compounds to investigate the role of the Ca\(^{2+}\) receptor in these responses largely circumvents these caveats. We have shown previously in rats that NPS R-568 completely blocks the rapid proliferative response of parathyroid cells to a partial nephrectomy (6). Moreover, if treatment with NPS R-568 is initiated within a day or so of the renal lesion, the animals do not develop 2\(^{\text{nd}}\)HPT as indicated by normal plasma levels of PTH and normal parathyroid gland histology (18). In the presence of the calcimimetic, the parathyroid cell is sensing a “hypercalcemic” condition and is not responding to the induced hypocalcemia. In the absence of alternative mechanisms, therefore, it is simplest to conclude that the effects of hypocalcemia on parathyroid cell growth are mediated by the Ca\(^{2+}\) receptor.

These earlier studies suggest that it might be possible to completely prevent 2\(^{\text{nd}}\)HPT in CRI if treatment with a calcimimetic compound is started very early and GFR has fallen only slightly. While identifying the predialysis patient and initiating preventative treatment may be a goal of the nephrologist, it is difficult and far from current practice. At present, patients presenting to the nephrologist have suffered significant loss of renal function and have already developed 2\(^{\text{nd}}\)HPT (5). Thus, the immediate problem is how to manage 2\(^{\text{nd}}\)HPT once it has developed and how to prevent it from progressing to a more severe form. This practical consideration has guided the design of the present experiments. The results obtained in these animal models of CRI show that it is possible to completely halt the progressive parathyroid gland enlargement once this process has begun.

In this study, the magnitude of the increase in total parathyroid gland volume was greatest and was progressive only in uremic rats fed the high phosphorus diet. This greater increase in gland size was not caused by differing degrees of CRI, because the elevations in plasma creatinine and BUN levels were very similar and relatively stable in both dietary groups. One of the hallmarks of renal 2\(^{\text{nd}}\)HPT, parathyroid cell hyperplasia, also occurred in this study. However, the role that cellular hypertrophy plays in parathyroid gland enlargement has seldom been investigated. These studies showed that increased average parathyroid cell volume contributed to glandular growth, but its role was minor compared with that of increased cell number, adding about 20% to the total increase.

### Table 1. Correlation analysis of parathyroid gland volume, average parathyroid cell volume, and total parathyroid cell number versus serum levels of PTH, calcium, phosphate, and 1,25(OH)\(_2\)D\(_3\).\(^a\)

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<tr>
<th>Variable</th>
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<th></th>
<th>Cell Volume</th>
<th></th>
<th>Cell Number</th>
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<td>Treated</td>
<td>Total</td>
<td>Untreated</td>
<td>Treated</td>
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<td></td>
<td>(&lt;0.001)</td>
<td>(&lt;0.001)</td>
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<td>(&lt;0.001)</td>
<td>(NS)</td>
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<td>0.073</td>
</tr>
<tr>
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<td>(NS)</td>
<td>(&lt;0.001)</td>
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<td>(NS)</td>
</tr>
<tr>
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</tr>
<tr>
<td></td>
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<td>(0.003)</td>
<td>(NS)</td>
</tr>
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\(^a\) The correlation coefficients for each analysis are shown with the corresponding P value in parentheses. Gland volume and cell number per kilogram were used in the analyses. Untreated animals include both sham-operated and CRI rats that received vehicle; treated animals were all CRI rats that received NPS R-568 by oral or subcutaneous routes. PTH, parathyroid hormone; CRI, chronic renal insufficiency.
in total gland volume. The mechanisms responsible for the hyperplasia and hypertrophy of parathyroid cells in CRI remain unclear. Although these studies were not designed to directly investigate the effects of plasma levels of Ca\(^{2+}\), phosphate, and 1.25(OH)\(_2\)D\(_3\) on hyperplasia and hypertrophy, the results did provide some insights. Parathyroid gland volume and total cell number were correlated equivalently with plasma levels of Ca\(^{2+}\) and phosphate, suggesting that both may play a role in the proliferative response. In contrast, no significant correlation was present with 1.25(OH)\(_2\)D\(_3\) levels, suggesting that the \textit{in vivo} effects of this hormone on cell proliferation may be mediated, at least in part, by the induced changes in plasma levels of Ca\(^{2+}\) (12). Correlations of average cell volume with plasma Ca\(^{2+}\) and phosphate levels were similar, suggesting that both may also play a role in the hypertrophy.

Although parathyroid gland volume and total cell number were strongly correlated with the plasma levels of phosphate, the administration of NPS R-568 completely prevented the progressive increase in gland volume and cell number that occurred in control CRI rats fed the high phosphorus diet, and did so despite a further increase in phosphate levels. The magnitude of this inhibitory effect on hyperplasia was greatest in the animals that received NPS R-568 by continuous subcutaneous infusion, although a significant effect also occurred with the high daily oral dose, which suppressed PTH levels only transiently. Thus, the Ca\(^{2+}\) receptor does not have to be activated chronically to completely halt the progression of parathyroid cell hyperplasia. Indeed, oscillations in circulating PTH levels rather than a sustained increase in phosphate levels. The magnitude of this inhibitory effect on hyperplasia was greatest in the animals that received NPS R-568 by continuous subcutaneous infusion, although a significant effect also occurred with the high daily oral dose, which suppressed PTH levels only transiently. Thus, the Ca\(^{2+}\) receptor does not have to be activated chronically to completely halt the progression of parathyroid cell hyperplasia. Indeed, oscillations in circulating PTH levels rather than a sustained suppression might be preferable under certain circumstances. For example, we have shown that daily transient decreases in PTH levels, but not the sustained suppression, increased bone mass in a rat model of CRI induced by adriamycin that is characterized by mild 2°HPT but low bone turnover (32).

In Experiment 1 in rats with mild 2°HPT, the infusion of NPS R-568 reduced total gland volume to that seen in sham-operated controls. This decrease in gland volume was caused solely by reduced cell volume. A significant decrease in cell volume below that present at the initiation of treatment was not seen with infused NPS R-568 in Experiment 2 in rats with more severe 2°HPT. Thus, NPS R-568 treatment prevented the progressive glandular growth and did so mainly by preventing further proliferation and, to a lesser extent, by reducing cell volume. However, in neither experiment did treatment with NPS R-568 reduce the number of cells below that present at the initiation of treatment.

It is important to note that these are static measures of parathyroid cell number taken at the end of the study and provide no dynamic information on cell turnover within the gland. We did not investigate whether NPS R-568 influenced parathyroid cell proliferation or apoptosis in these experiments. It is possible that the prevention of a further increase in total cell number by treatment with NPS R-568 could occur as a result of increased apoptosis that offsets a continued high proliferation rate. However, we consider that this is an unlikely explanation. Our earlier study in which we measured parathyroid cell proliferation directly showed that the rapid hyperplastic response to an acute subtotal nephrectomy was prevented by twice daily oral administration of NPS R-568 at a total daily dose of 100 \(\mu\)mol/kg (6). In that study we, like others (9), were unable to detect any parathyroid cells undergoing apoptosis, so such a process appears to be a very rare event in the rat.

In contrast, in a preliminary report from Driëcke’s group, apoptosis could be detected in normal human parathyroid tissue, and an increased frequency of apoptotic cells was observed in tissue from patients with 2°HPT (33,34). If calcimimetic agents completely block hyperplasia in patients with 2°HPT, as we showed in this and in other studies in rats (6,18), then continued (or accelerated) apoptosis in those patients will ultimately result in a decrease in parathyroid gland size in contrast to what occurred in our relatively short-term studies in rats.

The mechanisms linking the Ca\(^{2+}\) receptor to cellular growth are unknown, but they are specific to parathyroid cells, because other endocrine cells that alter hormone secretion in response to changes in the level of extracellular Ca\(^{2+}\) do not proliferate during short-term hypocalcemic conditions \textit{in vivo} (6). In addition to identifying the Ca\(^{2+}\) receptor as an important regulator of parathyroid cell growth, the results of our studies address the relative roles of the primary factors believed to be most important in the pathogenesis of 2°HPT. Significantly, progressive enlargement of the parathyroid glands can be halted even under conditions of chronic hyperphosphatemia and low levels of 1.25(OH)\(_2\)D\(_3\). Although phosphate and 1.25(OH)\(_2\)D\(_3\) clearly contribute to the regulation of parathyroid cell function, Ca\(^{2+}\), acting through the Ca\(^{2+}\) receptor, appears to play the dominant role. The results obtained with calcimimetic compounds in animal models of CRI complement the expanding literature, which is based on various experimental approaches showing that the Ca\(^{2+}\) receptor is the essential and primary molecular entity regulating several parathyroid cell functions in addition to secretion of PTH. In the aggregate, the results suggest that treatment with a calcimimetic compound could be sufficient to manage 2°HPT. We speculate that the induced fall in serum Ca\(^{2+}\) levels in the CRI patient treated with a calcimimetic agent can be prevented or reversed by dietary calcium supplementation, which will also help prevent hyperphosphatemia. If calcium supplementation alone fails to maintain Ca\(^{2+}\) levels within the normal range, then concomitant treatment with a calcemic vitamin D analog such as calcitriol may be necessary. Calcitriol treatment will also help control 2°HPT by inhibiting PTH synthesis (31).

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