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The Calcimimetic NPS R-568 Decreases Plasma PTH in Rats with Mild and Severe Renal or Dietary Secondary Hyperparathyroidism

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NPS R-568 is a Ca^{2+} receptor agonist ("calcimimetic") compound that reduces circulating parathyroid hormone (PTH) levels in rats and humans with mild secondary hyperparathyroidism (2ºHPT) resulting from chronic renal insufficiency (CRI). These studies extend those observations to show that NPS R-568 is equally effective in decreasing plasma PTH and Ca^{2+} levels in rats with mild or severe 2ºHPT, resulting either from CRI or from dietary calcium deficiency. Male rats were 5/6 nephrectomized and fed either normal chow or a high-phosphorus diet; other normal rats were fed a low-calcium diet. When 2ºHPT had developed, NPS R-568 was administered and blood samples were collected for up to 6 h. PTH levels decreased to a minimum level within 30 min in both CRI and calcium deficiency models of 2ºHPT. PTH and Ca^{2+} levels remained significantly depressed for >3 h after dosing. The percentage decrease in PTH levels was unaffected by the severity of 2ºHPT or the basal plasma Ca^{2+} or phosphate levels. In rats with severe 2ºHPT, the minimum plasma PTH level after NPS R-568 was greater than the basal level in mild 2ºHPT. Thus, NPS R-568 is equally effective in suppressing plasma PTH and Ca^{2+} levels in rats with mild or severe renal or nutritional 2ºHPT.

Key Words: Ca^{2+} receptor; calcimimetics; chronic renal insufficiency; dietary calcium deficiency; secondary hyperparathyroidism.

Introduction

Secondary hyperparathyroidism (2ºHPT) and renal osteodystrophy are common complications of chronic renal insufficiency (CRI). Current therapies used to treat 2ºHPT in CRI include 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], which inhibits parathyroid hormone (PTH) synthesis by decreasing transcription of the PTH gene and inhibits PTH secretion indirectly by increasing circulating ionized calcium (Ca^{2+}) levels. A second major therapy is the use of oral phosphate binders that decrease the intestinal absorption of phosphate and thereby reduce the magnitude of the hyperphosphatemia, an almost universal consequence of CRI. Hyperphosphatemia is thought to play a role in the pathogenesis of 2ºHPT both via direct effects on the parathyroid gland and by the hypocalcemia it induces *(1–6)*. Although newer phosphate binders are in development *(7,8)*, calcium salts are currently the most commonly used phosphate binders. However, their use in combination with $1,25(OH)_2D_3$ often results in hypercalcemia.

The regulation by extracellular Ca^{2+} of the secretion of PTH and calcitonin is mediated by a Ca^{2+} receptor that is expressed on the surface of parathyroid cells and C-cells *(9–13)*. The Ca^{2+} receptor may be a unique molecular target for drugs effective in treating hyperparathyroidism *(14)*. We have identified NPS R-568, a small orally active compound that acts as a positive allosteric modulator at the Ca^{2+} receptor *(15)*. Compounds that mimic or potentiate the effects of extracellular Ca^{2+} at the Ca^{2+} receptor have been termed "calcimimetics" *(14,15)*. When administered by gavage to normal rats, NPS R-568 induces hypocalcemia by inhibiting PTH secretion and, at higher doses, by stimulating the secretion of calcitonin *(16,17)*. Additional studies in thyroidectomized rats showed that the initial rapid fall in the plasma levels of Ca^{2+} is caused by the elevation in calcitonin levels, whereas the sustained hypocalcemic response is caused by the decrease in plasma PTH *(17)*. NPS R-568 has also been shown to decrease plasma PTH levels acutely in mild 2ºHPT both in rats with CRI *(18)* and in dialysis patients *(19)*.

The expression of the Ca^{2+} receptor in the parathyroid glands is reduced in patients with severe 2ºHPT, particularly in parathyroid tissue showing nodular hyperplasia *(20,21)*. A recent report has indicated that a similar downregulation of the parathyroid Ca^{2+} receptor may also occur in rats with severe uremic 2ºHPT; in that study, dietary phosphorus restriction prevented this receptor loss,

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suggesting that hyperphosphatemia may affect Ca^{2+} receptor expression *(22)*. It is therefore possible that, because of lower Ca^{2+} receptor levels, patients and animals with severe 2°HPT may be less responsive to the inhibitory actions of NPS R-568 on PTH secretion. The goals of these experiments were:

1. To extend the preliminary observations *(18)* that NPS R-568 decreases circulating PTH levels in rats with CRI and mild 2°HPT.
2. To assess whether NPS R-568 is effective in lowering the plasma levels of PTH and Ca^{2+} in hyperphosphatemic rats with severe 2°HPT.
3. To compare the responses in uremic rats with those seen in rats with mild and severe 2°HPT induced by dietary calcium deficiency.

Results

Studies in Rats with CRI and Mild 2°HPT

Blood urea nitrogen (BUN) and plasma PTH levels were elevated 3.3- and 2.8-fold, respectively, in the 5/6 nephrectomized (Nx) rats fed normal chow, confirming both the successful induction of CRI and the development of a mild 2°HPT. Basal plasma Ca^{2+} levels were also slightly, though significantly, lower in rats with CRI, whereas plasma phosphate and calcitonin levels were not elevated significantly. Body weight was unaffected by the uremic state (Table 1). The oral administration of NPS R-568 in these rats resulted in a rapid decrease in plasma PTH levels to a nadir at either 15 or 30 min postdose in both sham-operated and 5/6 Nx rats. The minimum plasma PTH level (4 ± 1 pg/mL) was the same in both groups of animals. PTH levels started to increase again by 1 h in both groups, but remained significantly lower than levels in the respective vehicle-dosed controls for 4 h in the sham-operated rats and throughout the experiment (6 h) in the 5/6 Nx rats (Fig. 1). Plasma levels of calcitonin increased rapidly after dosing and reached maximum levels at 15 or 30 min in both groups of rats. The maximum increase in calcitonin levels in rats given NPS R-568 was significantly ($p < 0.02$) greater in 5/6 Nx animals (290 ± 46 pg/mL) than in sham-operated controls (110 ± 32 pg/mL). Calcitonin levels declined rapidly from this peak, were not significantly different from respective vehicle-dosed controls at 60 min (sham-operated rats) or 90 min (5/6 Nx rats), and eventually fell slightly below basal levels (Fig. 1). Plasma levels of Ca^{2+} decreased

Table 1
Basal Parameters in Sham-Operated and 5/6 Nephrectomized Rats Fed Normal Rodent Chow[a]

	Sham-operated	5/6 Nephrectomized
Plasma		
BUN, mg/dL	19 ± 2	62 ± 12[b]
PTH, pg/mL	17 ± 3	47 ± 6[c]
Ca^{2+}, mmol/L	1.38 ± 0.02	1.32 ± 0.01[b]
Phosphate, mmol/L	2.19 ± 0.10	2.65 ± 0.45
Calcitonin, pg/mL	92 ± 12	128 ± 12
Body wt, g	349 ± 14	354 ± 6

[a]Body wt, Ca^{2+}, PTH, and calcitonin values are the averages of measurements taken on the two occasions that each rat was studied. BUN and phosphate levels are from blood collected at euthanasia. Values are means ± SEM $n = 5$/group.

[b]$p < 0.05$.

[c]$p < 0.01$: significance of difference from sham-operated controls.

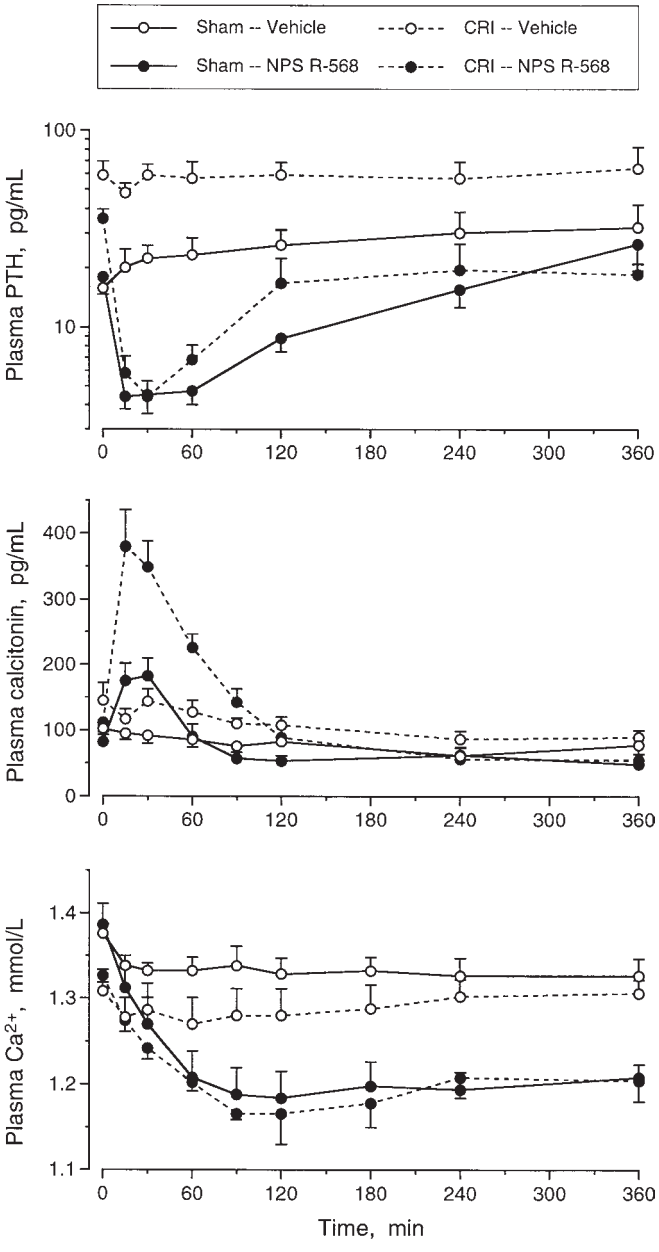

Fig. 1. Changes in plasma levels of PTH, calcitonin, and Ca^{2+} following the oral administration of vehicle or NPS R-568 (10 mg/kg) in conscious, sham-operated rats and in 5/6 nephrectomized (CRI) rats with mild 2°HPT. All rats were fed normal rodent chow containing 1.0% calcium, 0.7% phosphorus. Values are mean ±SEM $n = 4$–6/group.

promptly following NPS R-568 administration, were significantly depressed by 30 min in both groups, and reached a nadir at 90–120 min after dosing. There were no significant differences between sham-operated and 5/6 Nx rats in either the kinetics or the magnitude of the hypocalcemic response following the oral administration of NPS R-568. The maximum net decrement in plasma Ca^{2+} levels was 0.17 ± 0.01 and 0.16 ± 0.02 mmol/L in sham-operated and 5/6 Nx rats, respectively. Plasma Ca^{2+} then started to increase from this nadir, but levels for both groups remained significantly lower than those in respective control rats that received vehicle throughout the study (Fig. 1).

Studies in Rats with CRI and Severe 2°HPT

The plasma PTH response to systemically administered NPS R-568 was assessed in five 5/6 Nx rats fed a high-phosphorus diet in which the predose PTH levels varied from 33 to 1064 pg/mL (normal = 10–25 pg/mL). Plasma phosphate was raised in parallel with the increases in PTH levels, although hypocalcemia was present only in the rat with the second highest PTH level (Fig. 2). PTH levels decreased rapidly in all rats after the intra-arterial injection of NPS R-568, and reached a nadir at either 10 or 20 min postdose. The lowest PTH level achieved depended on the initial plasma PTH concentration. In the two rats with the highest basal plasma PTH, the nadir was higher than the predose PTH level in the rat with the lowest baseline value. However, when the plasma PTH level was expressed relative to the predose level of hormone in each rat, the maximum decrease in PTH levels induced by NPS R-568 injection was similar (82–94%) in all animals (Fig. 2). Plasma levels of Ca^{2+} decreased rapidly after the injection of NPS R-568 and were continuing to fall when the study ended. The percentage decrease in plasma Ca^{2+} levels was greatest in the two rats with the most severe 2°HPT and the lowest baseline Ca^{2+} levels. Phosphate levels also decreased in all rats; the average decrease at 30 min after the injection was 13 ± 2% (Fig. 2).

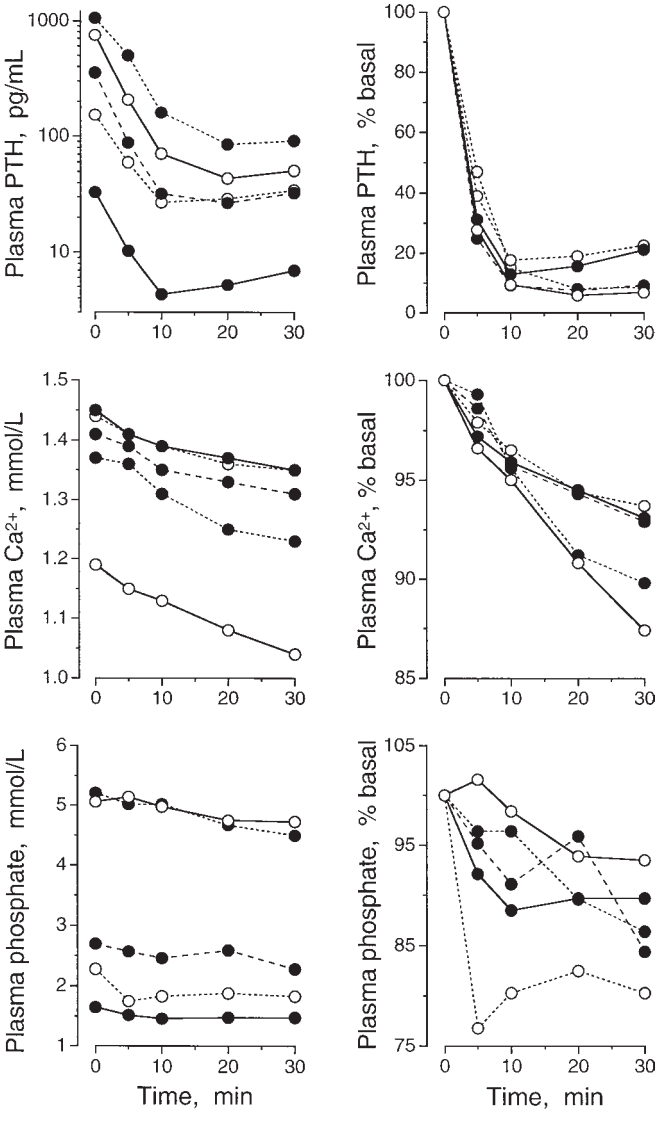

Fig. 2. Changes in plasma PTH, Ca^{2+}, and phosphate levels following the intraarterial injection of NPS R-568 (5 mg/kg) in five conscious, 5/6 nephrectomized rats fed a high-phosphorus diet (0.6% calcium, 0.8% phosphorus) and with widely differing magnitudes of 2°HPT. The percent decreases in PTH, Ca^{2+}, and phosphate levels from basal in each rat are shown in the right panels.

Studies in Rats with Dietary Calcium Deficiency

Short-Term Study

Basal plasma PTH levels averaged 2.3-fold higher ($p < 0.01$) in rats fed the calcium-deficient diet for 2–3 wk before study, but plasma Ca^{2+} levels were not significantly lower (Fig. 3). The oral administration of NPS R-568 significantly decreased plasma PTH in both dietary groups. A nadir in PTH levels occurred between 15 and 60 min in both normal and calcium-deficient rats. The maximum decrease in PTH levels was not significantly different between normal (87 ± 2%) and calcium-deficient rats (75 ± 8%). Plasma PTH remained significantly depressed below levels in respective vehicle-dosed controls for 3 h in both groups. Although the average plasma PTH concentration tended to be maintained at a higher level in calcium-deficient rats after the administration of NPS R-568 when compared to normal rats, the difference was not significant at any time-point ($p \geq 0.09$). The pattern and magnitude of the hypocalcemic response to orally administered NPS R-568 were also similar in rats fed either the normal or low-calcium diets (Fig. 3). In normal rats, plasma Ca^{2+} levels were significantly lower in rats given NPS R-568 than in vehicle-dosed controls from 30 min until the end of the study (6 h). In calcium-deficient rats, plasma Ca^{2+} was significantly suppressed from 30 to 180 min; this apparently shorter duration of hypocalcemia was, however, caused by a greater decrease in plasma Ca^{2+} levels in the calcium-deficient rats that received vehicle. There were no significant differences in plasma Ca^{2+} levels between normal and calcium-deficient rats at any time-point after NPS R-568 administration.

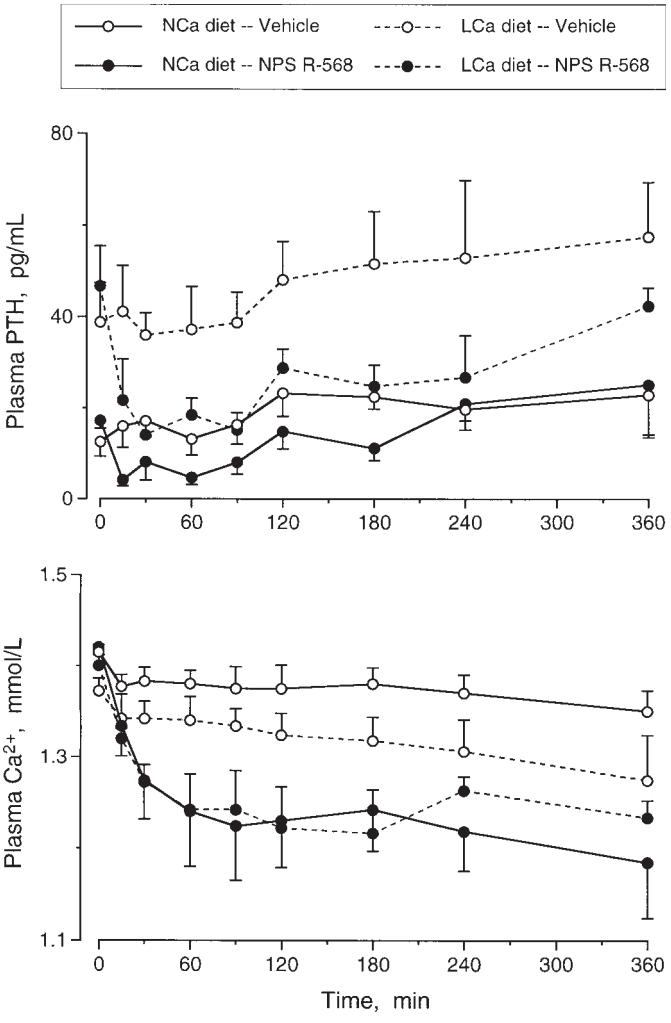

Fig. 3. Changes in plasma PTH and Ca^{2+} levels following the oral administration of vehicle or NPS R-568 (10 mg/kg) to conscious rats fed a normal calcium diet (0.8% calcium, 0.5% phosphorus) or to rats with mild 2°HPT induced by feeding a low-calcium diet (0.02% calcium, 0.5% phosphorus) for 2–3 wk prior to study. Values are mean ±SEM n = 5–6/group.

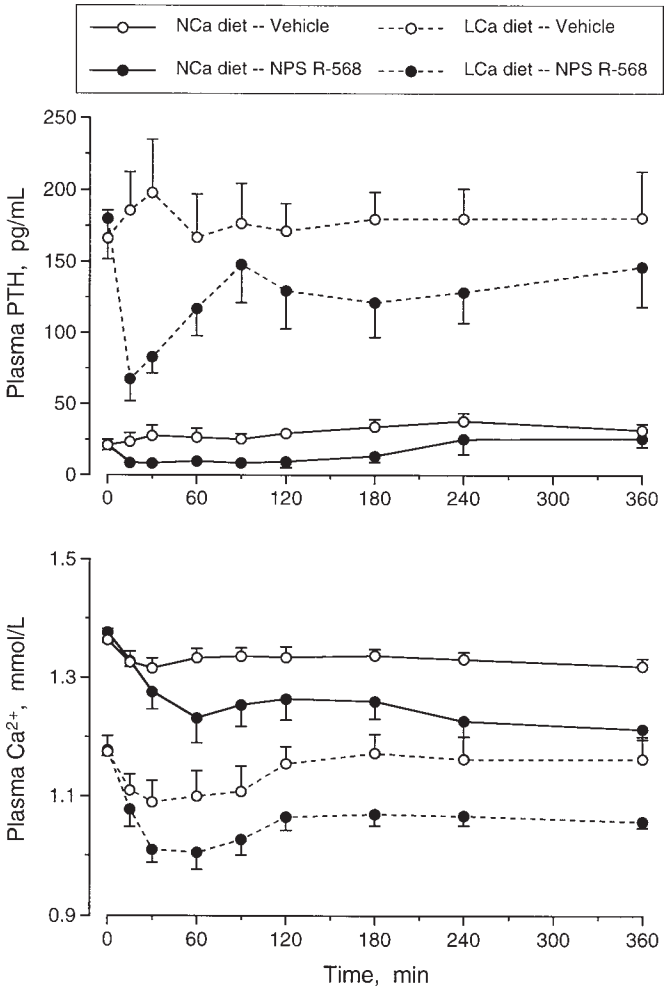

Fig. 4. Changes in plasma PTH and Ca^{2+} levels following the oral administration of vehicle or NPS R-568 (10 mg/kg) to conscious rats fed a normal calcium diet (0.8% calcium, 0.5% phosphorus) or to rats with severe 2°HPT induced by feeding a low-calcium diet (0.02% calcium, 0.5% phosphorus) for 5–6 wk prior to study. Values are mean ±SEM n = 5–7/group.

Long-Term Study

To induce a more severe 2°HPT, in the second experiment, the rats were fed the low-calcium diet for 5-6 wk before study. Basal plasma PTH and Ca^{2+} levels averaged 7.4-fold higher and 0.21 mmol/L lower ($p < 0.001$), respectively, in the calcium-deficient rats (Fig. 4). The oral administration of NPS R-568 caused a rapid decrease in plasma PTH to a minimum level at 15–60 min after dosing in both dietary groups. Although the percentage decrease in PTH levels after NPS R-568 administration was similar in rats fed the normal or low-calcium diets (69 ± 7 and 63 ± 6%, respectively), the minimum plasma PTH level achieved in calcium-deficient rats (65 ± 15 pg/mL) remained more than three times higher ($p < 0.05$) than basal levels in rats fed the normal calcium diet (Fig. 4). In the calcium-deficient rats, the plasma PTH concentration increased rapidly from this nadir and, apart from the 180 min time-point, was not significantly different from levels in vehicle-dosed controls from 60 to 360 min. In contrast, PTH levels in the calcium-replete animals remained significantly suppressed until 240 min after dosing. Plasma levels of Ca^{2+} decreased rapidly after NPS R-568 administration in both groups of rats, were significantly lower by 30 and 60 min in the calcium-deficient and normal rats, respectively, and reached a nadir at 60–90 min after dosing. Ca^{2+} levels tended to increase from this nadir in both groups, but remained significantly below concentrations in vehicle-dosed controls throughout the remainder of the study (Fig. 4).

Discussion

We have previously reported that the plasma levels of PTH and Ca^{2+} are lower at 1 h after the oral administration of NPS R-568 to partially nephrectomized rats with mild

2ºHPT *(18)*. These experiments confirmed those observations and extended them to show:

1. That plasma PTH and Ca^{2+} levels are suppressed for more than 6 h after the oral administration of NPS R-568 to uremic rats with a similar magnitude of 2ºHPT to those studied previously.
2. That NPS R-568 is equally effective at decreasing PTH levels and inducing hypocalcemia in rats with severe 2ºHPT resulting from CRI, despite the presence of severe hyperphosphatemia.
3. That NPS R-568 induces quantitatively similar changes in PTH and Ca^{2+} levels in rats with mild or severe 2ºHPT resulting from dietary calcium deficiency.

Plasma levels of PTH were elevated two- to fourfold in 5/6 Nx rats fed normal chow, but decreased to similar levels in both sham-operated and 5/6 Nx rats after the administration of NPS R-568. Moreover, there were no significant differences in the kinetics of the changes in plasma PTH and Ca^{2+} levels after dosing. The Ca^{2+} receptor is also expressed in the kidney where it may play a role in the regulation of tubular calcium reabsorption *(23)*. The administration of NPS R-568 to patients with primary hyperparathyroidism increases urinary Ca excretion *(24)*, but there is no evidence that this is attributable to anything other than the induced decrease in PTH levels. Indeed, we have shown that the kinetics and magnitude of the hypocalcemic response are unaffected by an acute total nephrectomy of rats, but are abolished by parathyroidectomy *(16)*, suggesting that the kidneys play a minimal, if any, role in the hypocalcemic response to NPS R-568.

These observations in 5/6 Nx rats fed chow confirm that the 2ºHPT was relatively mild in these animals. In contrast, PTH levels were variable in 5/6 Nx rats fed the high-phosphorus diet, with PTH levels 20–50 times normal in some animals. NPS R-568 administration decreased PTH levels markedly irrespective of the severity of the 2ºHPT or the magnitude of the hyperphosphatemia, but the minimum PTH level that occurred after dosing was dependent on the basal level. Indeed, in the two animals with the highest plasma PTH concentration, the lowest PTH level achieved was greater than the basal PTH level in the uremic rat with the least severe 2ºHPT. Thus, NPS R-568 decreased plasma PTH levels by approximately the same percentage in all animals despite the possibility that parathyroid Ca^{2+} receptor expression may be reduced in these animals *(22)*. The administration of NPS R-568 to rats with severe 2ºHPT also decreased phosphate levels. Although phosphate levels were not measured in the other experiments reported here, other studies in normal rats have shown that phosphate levels decrease for 30–60 min and then increase above baseline for about 2 h before returning back to predose levels by 4 to 6 h *(16)*. This acute decrease in plasma phosphate can be attributed to inhibition of osteoclast-mediated bone resorption, a result of increased calcitonin levels *(17)*, whereas the subsequent increase in plasma phosphate is presumably a consequence of decreased PTH levels and decreased urinary phosphate excretion.

In vitro results with dispersed bovine parathyroid cells have shown that NPS R-568 inhibits PTH secretion solely by its interaction with the Ca^{2+} receptor *(15)*. In those cells, NPS R-568 decreased PTH secretion by exactly the same amount as elevated extracellular Ca^{2+} concentrations. Thus, NPS R-568 did not inhibit the constitutive, calcium nonsuppressible component of PTH secretion *(25)*. As parathyroid cell hyperplasia progresses during the development of severe 2ºHPT, more PTH is secreted constitutively. However, because the percentage decrease in PTH levels in the current study was the same irrespective of the basal PTH concentration, this suggests that in renal 2ºHPT the proportion of PTH secretion that is constitutive remains the same as from normal parathyroid glands.

Of interest was the observation that basal PTH levels in 5/6 Nx rats fed the high-phosphorus diet were better correlated ($r = 0.97$) with plasma phosphate levels than with plasma Ca^{2+} or with BUN levels. The concept that phosphate is an important regulator of parathyroid gland function has been advanced by Slatopolsky and colleagues for many years, and our current data appear to agree with this hypothesis *(3,6,26)*. However, our data also show that activation of the Ca^{2+} receptor with a calcimimetic compound can overwhelm any stimulatory effects of phosphate on PTH secretion.

Although the mRNA coding for the Ca^{2+} receptor is identical in C-cells and parathyroid cells *(12,13)*, the pharmacological sensitivity of the two receptors differs. NPS R-568 exhibits about a 40-fold greater potency in reducing PTH levels than in increasing the plasma levels of calcitonin *(16,17)*. Previous experiments showing that the 10 mg/kg dose of NPS R-568 causes a transient twofold increase in calcitonin levels in normal rats were confirmed in this study. However, the plasma calcitonin response was almost twofold greater in rats with CRI. Although we cannot exclude the possibility that this exaggerated response was caused by increased calcitonin secretion, it is more likely a result of reduced clearance of the hormone, since the kidney is the most important site of calcitonin catabolism *(27)*. Despite the larger calcitonin response to NPS R-568 in rats with CRI, the hypocalcemic response was virtually identical in both groups. This suggests that the average maximum 2.7-fold increase in calcitonin levels in sham-operated rats is sufficient to induce a maximal hypocalcemic response. Such a conclusion is supported by earlier studies that showed that the rate of onset of hypocalcemia was the same in normal rats receiving 10–100 mg/kg oral doses of NPS R-568, which caused 2- to 10-fold increases in the plasma levels of calcitonin. In contrast, the rate of onset of hypocalcemia was considerably slower in normal rats receiving 3.3 mg/kg NPS R-568, a dose that maximally inhibited PTH secretion, but did not affect the plasma levels of calcitonin *(16,17)*.

NPS R-568 also reduced plasma levels of PTH and Ca^{2+} in rats with both mild and severe 2ºHPT resulting from dietary calcium deficiency. These changes occurred irrespective of whether or not the animals were hypocalcemic at the initiation of the study. The kinetics and the magnitude of the changes in PTH and Ca^{2+} levels were similar in calcium-replete and calcium-deficient rats, and were also similar to those in rats with CRI. As was seen in the rats with severe renal 2ºHPT, the minimum plasma PTH level achieved after NPS R-568 administration in calcium-deficient rats with severe 2ºHPT was higher than the basal PTH concentration in control rats fed the normal calcium diet. This indicates that marked parathyroid cell hyperplasia with increased constitutive PTH secretion also occurred in this animal model.

There are several important differences between the two models of 2ºHPT. For example, plasma phosphate usually is elevated in rats with CRI, but because PTH is phosphaturic, phosphate levels tend to be low in calcium-deficient rats *(28)*. However, the studies in rats with CRI described above show that the magnitude of the decrease in PTH levels induced by NPS R-568 is unaffected by the prevailing plasma phosphate concentrations. Plasma $1,25(OH)_2D_3$ levels are elevated as much as 15-fold in severely calcium-deficient rats *(28)*, whereas $1,25(OH)_2D_3$ levels are reduced in rats with CRI *(18,29)*. The injection of large doses of $1,25(OH)_2D_3$ increases Ca^{2+} receptor expression in the parathyroid glands of normal rats *(30)*. This upregulation has been proposed, at least in part, as the mechanism responsible for the increased sensitivity of PTH secretion to inhibition by plasma Ca^{2+} in dialysis patients treated with $1,25(OH)_2D_3$ *(31)*. However, we found no effects on parathyroid gland Ca^{2+} receptor expression when circulating $1,25(OH)_2D_3$ levels were increased chronically either by infusion of the hormone or by dietary calcium deficiency *(32,33)*.

In conclusion, the calcimimetic compound NPS R-568 is equally effective in suppressing plasma levels of PTH in rats with CRI- and dietary-induced 2ºHPT, despite the marked differences between the two models. Thus, calcimimetic compounds like NPS R-568 are potentially useful therapeutic agents in the treatment of hyperparathyroidism.

Materials and Methods

Studies in Rats with CRI

Male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), weighing about 250 g, were housed in hanging wire cages for at least 7 d prior to surgery. They were provided with unrestricted access to tap water and a commercial rodent chow (Purina 5001), which contained 1.0% calcium and 0.7% phosphorus. The rats were anesthetized with ketamine and xylazine, and subjected either to a one-stage 5/6 Nx or to a sham operation as described previously *(33)*. The rats were studied 6–8 wk after surgery, which allowed CRI and mild 2ºHPT to develop. The rats were reanesthetized at least 2 d before study, and a chronic indwelling polyvinyl catheter was implanted into the abdominal aorta via the femoral artery *(34)*. All experimental procedures were approved by the Institutional Animal Care and Use Committee of NPS Pharmaceuticals, Inc.

Most rats were studied on two occasions in a crossover design. First, half of the rats received vehicle, a 1.5% aqueous solution of 2-hydroxypropyl-β-cyclodextrin (Research Biochemicals, Natick, MA), administered by gavage in a volume of 1.0 mL/200 g body wt. The other half received NPS R-568 (10 mg/kg) as the hydrochloride salt (9 mg/kg as free base; 30 μmol/kg). Four or 5 d later, each rat received the opposite treatment. Blood samples (0.8 mL) were collected into a heparinized syringe for measurement of plasma Ca^{2+}, PTH, and calcitonin levels before and at 15, 30, 60, 90, 120, 180, 240, and 360 min after dosing. To prevent excessive blood loss associated with the frequent sampling, the red cell pellet from each blood sample was resuspended in an equal volume of normal rat plasma and reinjected. At euthanasia, 1 wk after the second study, plasma was collected for assay of BUN and phosphate levels.

To assess the plasma PTH and Ca^{2+} responses to NPS R-568 in rats with severe 2ºHPT, one group of 5/6 Nx rats was fed a semisynthetic, high-phosphorus diet (0.6% calcium, 0.8% phosphorus; TD95211, Teklad, Madison, WI). After 11 diet weeks, a blood sample was collected from the tail, and five rats with widely variable plasma PTH levels were selected for study. An arterial catheter was implanted in each animal, and 4–5 d later, NPS R-568 (5 mg/kg) was injected *via* that catheter. Plasma levels of Ca^{2+}, PTH, phosphate, and BUN were measured in the predose sample; additional samples were collected at 5, 10, 20, and 30 min after the injection for PTH, Ca^{2+}, and phosphate analyses.

Studies in Rats with Dietary Calcium Deficiency

Two separate experiments were performed. In both experiments, normal male Sprague-Dawley rats were fed semisynthetic diets (Teklad) with either a normal (0.8% calcium, 0.5% phosphorus; TD87092) or a low-calcium content (0.02% calcium, 0.5% phosphorus; TD88050). In the first experiment, the rats weighed 175–200 g on receipt and were fed the diets for 2 wk before study. To induce a more severe 2ºHPT, in the second experiment, the rats weighed 100–125 g on receipt and the diets were fed for 5–6 wk. An arterial blood sampling catheter was implanted at least 2 d before study in all rats. The experimental protocol was identical to that described above for the 5/6 Nx rats with mild 2ºHPT, except that the plasma levels of calcitonin were not measured in these studies.

Analyses

Plasma Ca^{2+} levels were measured immediately after sample collection using a model 634 Ca^{2+} analyzer (Ciba Corning, Medford, MA). Plasma levels of PTH were measured with a two-site rat PTH-(1-34) immunoradiometric

assay (Immutopics, San Clemente, CA). Plasma calcitonin levels were measured by radioimmunoassay using goat antihuman calcitonin antiserum G-813, high-performance liquid chromatography-purified ^{125}I-human calcitonin tracer, and rat calcitonin standards *(35)*. BUN and phosphate levels were measured using a multichannel analyzer (Monarch 1000; Instrumentation Laboratory, Lexington, MA).

All data are presented as means ± SEM and were initially subjected to analysis of variance (ANOVA) for repeated measures (SuperANOVA, Abacus Concepts, Berkeley, CA). Fisher's protected least-significant-difference multiple-comparison test was used to compare values at each time-point. A *t*-test or the Mann–Whitney U-test (where the variances were unequal) was used to compare basal values between sham-operated and 5/6 Nx rats or between normal and calcium-deficient rats.

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