View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 58 (2000), pp. 753–761

A novel mechanism for skeletal resistance in uremia

EDUARDO SLATOPOLSKY, JANE FINCH, PATRICIA CLAY, DANIEL MARTIN, GREGORIO SICARD, GARY SINGER, PING GAO, THOMAS CANTOR, and ADRIANA DUSSO

Renal Division, Washington University Medical School, St. Louis, Missouri, and Scantibodies Laboratory, Santee, California, USA

A novel mechanism for skeletal resistance in uremia.

Background. In treating secondary hyperparathyroidism, the target level of serum intact parathyroid hormone (I-PTH) should be three to five times normal to prevent adynamic bone disease. In circulation, there is a non-(1-84) PTH-truncated fragment, likely 7-84, which, in addition to PTH 1-84, is measured by most I-PTH immunoradiometric (IRMA) assays, giving erroneously high I-PTH values. We have developed a new IRMA assay in which the labeled antibody recognizes only the first six amino acids of the PTH molecule. Thus, this new IRMA assay (Whole PTH) measures only the biologically active 1-84 PTH molecule.

Methods. Using this new IRMA assay (Whole PTH) and the Nichols "intact" PTH assay, we compared the ability of each assay to recognize human PTH (hPTH) 1-84 and hPTH 7-84 and examined the percentage of non-1-84 PTH in circulation and in parathyroid glands. Possible antagonistic effects of the 7-84 PTH fragment on the biological activity of 1-84 PTH in rats were also tested.

Results. In 28 uremic patients, PTH values measured with the Nichols assay, representing a combined measurement of both hPTH 1-84 and hPTH 7-84, were 34% higher than with the Whole assay (hPTH 1-84 only); the median PTH was 523 versus 318 pg/mL ($P < 0.001$). Similar results were found in 14 renal transplant patients. In osteoblast-like cells, ROS 17.2, 1-84 PTH (10^{-8} mol/L) increased cAMP from 18.1 ± 1.25 to 738 ± 4.13 mmol/well. Conversely, the same concentration of 7-84 PTH had no effect. In parathyroidectomized rats fed a calcium-deficient diet, 7-84 PTH was not only biologically inactive, but had antagonistic effects on 1-84 PTH in bone. Plasma calcium was increased (0.65 mg/dL) two hours after 1-84 PTH treatment, while 7-84 PTH had no effect. When 1-84 PTH and 7-84 PTH were given simultaneously in a 1:1 molar ratio, the calcemic response to 1-84 PTH was decreased by 94%. In normal rats, the administration of 1-84 PTH increased renal fractional excretion of phosphate (11.9 to 27.7%, $P < 0.001$). However, when 1-84 PTH and 7-84 PTH were given simultaneously, the 7-84 PTH decreased the phosphaturic response by 50.2% ($P < 0.005$). Finally, in surgically excised parathyroid glands from six uremic patients, we found that 44.1% of the total intracellular PTH was the non-PTH (1-84), most likely PTH 7-84.

Conclusion. In patients with chronic renal failure, the presence of high circulating levels of non–1-84 PTH fragments (most likely 7-84 PTH) detected by the "intact" assay and the antagonistic effects of 7-84 PTH on the biological activity of 1-84 PTH explain the need of higher levels of "intact" PTH to prevent adynamic bone disease.

Key words: adynamic bone disease, IRMA for PTH, parathyroid, chronic renal failure, secondary hyperparathyroidism.

Received for publication November 11, 1999
and in revised form February 7, 2000
Accepted for publication March 2, 2000

© 2000 by the International Society of Nephrology

Parathyroid hormone (PTH), a single-chain polypeptide of 84 amino acids [1], plays a critical role in the regulation of mineral metabolism. Ionized calcium, calcitriol, and phosphorus are the three major regulators of PTH homeostasis in humans.

The human PTH gene is located on the short arm of chromosome 11. The coding region spans more than 4 kb and consists of three exons. The first exon contains the 5′ untranslated region of the PTH transcript. The coding sequence spans exons 2 and 3. The spliced cytoplasmic transcript is 772 bases long. The primary translation product, pre-pro-PTH (115 amino acids), is formed in the rough endoplasmic reticulum of parathyroid chief cells [2] and is converted within seconds to pro-PTH (90 amino acids) [3]. In the Golgi apparatus, pro-PTH is converted to intact PTH (I-PTH; 84 amino acids) approximately 15 minutes after the biosynthesis of the original pre-pro-PTH and approximately 50 minutes prior to PTH secretion [3, 4].

Parathyroid hormone (1-84) is stored in secretory granules awaiting one of two fates: secretion in response to hypocalcemic stimuli or intracellular degradation. Intracellular PTH degradation increases in states of hypercalcemia when PTH secretion rates are lower. In contrast, the absolute rate of hormone degradation decreases in hypocalcemic conditions when the secretion of PTH 1-84 is enhanced [5–8].

Secondary hyperparathyroidism is a universal finding in patients with chronic renal failure. The pathological consequences of high levels of PTH are well known [9, 10] and have a significant impact on the life of patients with chronic renal insufficiency. Thus, accurate monitor-

ing of the levels of biologically active PTH is of great importance for the appropriate treatment of secondary hyperparathryoidism in these patients.

The development of immunoradiometric (IRMA) and immunochemiluminometric (ICMA) two-site assays for human I-PTH have greatly improved the monitoring of PTH levels in patients with chronic renal failure [11–15]. These commercial assays, also known as sandwich assays, use two immunoaffinity-purified antibodies. One antibody, immobilized on a solid support, often a plastic surface, serves as a "capture" antibody and binds the C-terminal portion of the I-PTH, as well as midregion epitopes. A second antibody, labeled with radioactive iodine, in IRMA assays binds the N-terminal region of the 1-84 PTH molecule. The development of these assays has simplified the interpretation of measurements for PTH since theoretically they detect only PTH 1-84, or I-PTH, which is biologically active. The midregion and C-terminal–circulating fragments, which are greatly increased in renal insufficiency [16–21], are usually not detected by these assays [11]. However, Quarles, Lobaugh, and Murphy demonstrated that in patients with renal failure, the measurement of "intact" PTH overestimates the overall degree of secondary hyperparathyroidism, since levels of "intact" PTH, which are two to three times above the normal levels, correlate with normal bone histology while normal levels of "intact" PTH in these patients are associated with adynamic bone disease [22]. Therefore, when utilizing commercial assays for I-PTH, it is necessary to maintain the levels of PTH above those of normal individuals to prevent adynamic bone disease. This initial observation has been confirmed by several investigators [23], and currently, it is an accepted practice to maintain serum I-PTH at levels three to five times (200 to 300 pg/mL) above normal values to prevent adynamic bone disease.

Lepage et al demonstrated that when serum from uremic patients is fractionated by high-performance liquid chromatography (HPLC), two immunoreactive peaks can be detected by the Nichols two-site I-PTH assay [24]. According to these investigators, one peak was shown to comigrate with synthetic human PTH (hPTH) 1-84, and a second more hydrophilic peak, eluting slightly ahead of hPTH 7-84, accumulates in renal failure and accounts for 40 to 60% of the total immunoreactivity in these patients compared with only 10 to 20% in healthy individuals [25]. Clearly, a non-(1-84) PTH fragment, "likely" containing amino acids 7-84 of the PTH molecule, circulates in serum and is measured by most commercial "intact" assays [24].

The presence of circulating inhibitors of PTH in uremic patients has been proposed as one of the causes for the need for higher levels of PTH to maintain normal bone histology (abstracts; Colford et al, *10th International Congress of Endocrinology*, San Francisco, The International Society of Endocrinology and the Endocrine Society, Minneapolis, 1996; and Colford et al, *79th Annual Meeting of the Endocrine Society*, Minneapolis, 1997) [25]. Thus, the purpose of this study was to characterize a new IRMA ("Whole" PTH assay), which, in contrast to commercially available "intact" assays, recognizes only intact human PTH 1-84, and to examine the potential of hPTH 7-84 to inhibit the biological activity of hPTH 1-84.

METHODS

Characterization of the new "Whole" PTH IRMA assay

This new Whole PTH two-site assay (Scantibodies Laboratories, Santee, CA, USA) first employs an antibody that recognizes the 39-84 region of the PTH molecule. This antibody, produced in a goat and affinity purified, is present in relative excess and is immobilized onto polystyrene-coated tubes. The second antibody, also developed in a goat, was also affinity purified and recognizes only the first six amino acids (1 to 6; Ser-Val-Ser-Glu-Ile-Gln) of the human PTH molecule (Fig. 1). This anti-hPTH (1 to 6) antibody is labeled with ^{125}I. The Whole PTH assay uses synthetic human PTH 1-84 as the standard, with a limit of detection of approximately 1 to 2 pg/mL. Normal values range from 5 to 35 pg/mL. The interassay and intra-assay coefficients of variation were found to be between 2 and 7%, and recovery was from 96 to 106%. The Whole PTH assay was compared with the Intact PTH assay purchased from the Nichols Institute (I-Nichols, San Juan Capistrano, CA, USA). Synthetic human PTH 1-84 and 7-84 were purchased from Bachem (Torrance, CA, USA). To assess circulating levels of hPTH 1-84 and non-(1-84) PTH, heparinized blood samples were obtained before dialysis from 28 patients who had been maintained on chronic hemodialysis for 1.2 to 7.5 years and from 14 renal transplant patients (1 to 6 years).

Studies in vitro

Osteoblastic cell line. To compare the biological effects of the two peptides (hPTH 1-84 and 7-84), intracellular cAMP production was measured in the rat osteosarcoma cell line ROS/17.2, which has an osteoblastic phenotype and is known to increase cAMP production in response to PTH. Cells were cultured in Ham's F12 media containing 10% fetal bovine serum. Cells were plated out in 12-well plates at a density of 30,000 cells per well and grown to confluence. Cells were washed three times with KHMS buffer at 37°C (KCl 4.0 mmol/L, $CaCl_2$ 1.25 mmol/L, $MgSO_4$ 1.25 mmol/L, KH_2PO_4 1.2 mmol/L, HEPES 10 mmol/L, NaCl 100 mmol/L, $NaHCO_3$ 37 mmol/L, and glucose 10 mmol/L, pH 7.5). cAMP production was measured using 500 μL of KHMS buffer

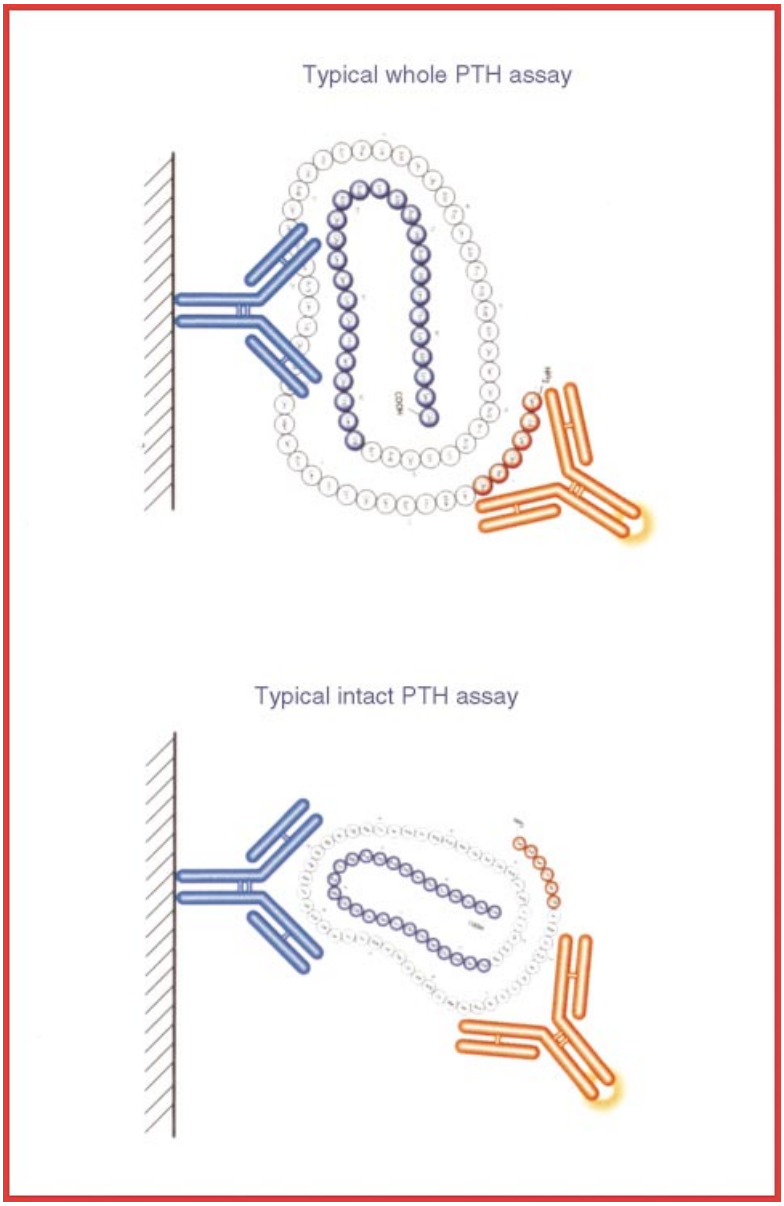

Fig. 1. Immunodetection of parathyroid hormone (PTH) by the Whole PTH and Nichols Intact PTH (I-PTH) assays. In both assays, the immobilized antibody recognizes a large portion of the C/midregion of the PTH molecule. However, in the Whole PTH assay (top), the labeled antibody recognizes only the first six amino acids, while the labeled antibody from the typical I-PTH assay (bottom) recognizes a larger number of amino acids in the N-terminal region.

(37°) containing isobutyl-1-methylxantine (IBMX) 1.0 mmol/L and various concentrations (10^{-11} to 10^{-8} mol/L) of hPTH 1-84 or hPTH 7-84. After a five-minute incubation, 100 μL of 1.8 mol/L perchloric acid were added. After an additional five-minute incubation at room temperature, 100 μL of 3 mol/L $KHCO_3$ were added to neutralize the acid. Samples were centrifuged at 3000 rpm for 15 minutes, and the supernatants were assayed for cAMP [26].

Analysis of PTH in human parathyroid glands. Human parathyroid glands were placed in ice-cold phosphate-buffered saline and processed within 30 minutes of parathyroidectomy. Aliquots of parathyroid tissue were dissected, weighed, and homogenized in 500 μL of a buffer containing 100 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 1 mol/L DL-dithiothreitol, and a complete TM protease inhibitor cocktail (Boehringer-Mannheim, Mannheim, Germany). Homogenates were sonicated three times for 30 seconds each at 0°C and centrifuged at 10,000 × *g* for 15 minutes. Supernatants were kept at −70°C until measurements of 1-84 PTH, non-(1-84) PTH, and total protein were performed.

Studies in vivo

Calcemic response. Normal female Sprague-Dawley rats weighing 225 to 250 g (Harlan, Indianapolis, IN, USA) were parathyroidectomized (PTX) and fed a 0.02% calcium diet. Rats with a plasma calcium below

7.0 mg/dL after overnight fasting were included in the study. A 20 μg dose of hPTH 1-84 or 7-84 was given intraperitoneally to PTX rats in four doses of 5 μg each at 30-minute intervals (0, 30, 60, and 90 minutes). For control studies, the rats received vehicle (saline solution) alone. Blood was drawn via the tail at 0, 60, 90, and 120 minutes. For competition experiments, rats received an injection of hPTH 7-84 10 minutes prior to each injection of hPTH 1-84. The molar ratio of hPTH 7-84:hPTH 1-84 was 1:1.

Phosphaturic response. Normal female Sprague-Dawley rats weighing 225 to 250 g were prepared for clearance studies under light anesthesia. Polyethylene catheters (PE50) were placed in the femoral artery for the collection of blood and measurement of blood pressure (Blood Pressure Analyzer; Micro-Medic, Inc., Louisville, KY, USA), in the femoral vein for infusion and in the bladder for the collection of urine. Rats were placed in Plexiglas® holders and allowed to recover from the effect of the anesthetic for one hour. A priming dose (0.6 mL) of chemical inulin in saline was administered over a period of three minutes to achieve a plasma inulin level between 50 and 100 mg/mL. A solution of saline containing inulin to maintain this level and calcium gluconate to deliver 0.5 mg calcium was infused at the rate of 0.03 mL/min. After equilibration, a total of four 30-minute urine collections was obtained.

To assess the effects of hPTH 1-84 on phosphate excretion, urine was collected during two control periods, after which rats received a priming bolus of 1.8 μg of hPTH 1-84 followed by a sustained infusion that delivered a total of 8.2 μg of I-PTH. After an equilibration period of 20 minutes, two 30-minute urine collections were obtained. In competition experiments, hPTH 7-84 was given five minutes prior to hPTH 1-84 at a molar ratio of 4:1.

Blood samples and blood pressure measurements were recorded at the beginning and end of the baseline periods, at the beginning of the PTH infusion period, and at the end of the study. The concentration of inulin in plasma and urine was determined by the method of Führ, Kaczmarczyk, and Kruttgen [27]. The estimation of the glomerular filtration rate (GFR) by inulin clearance and the calculation of the fractional urinary excretion rate of phosphorus (FE_{PO4}) were performed in the standard fashion. Blood samples were centrifuged, and plasma phosphorus and calcium concentrations were measured.

Serum chemistries

Total plasma calcium levels were determined using an atomic absorption spectrophotometer (model 1100B; Perkin Elmer, Norwalk, CT, USA). Plasma phosphorus levels were determined using an autoanalyzer (COBAS MIRA Plus; Roche, Newark, NJ, USA).

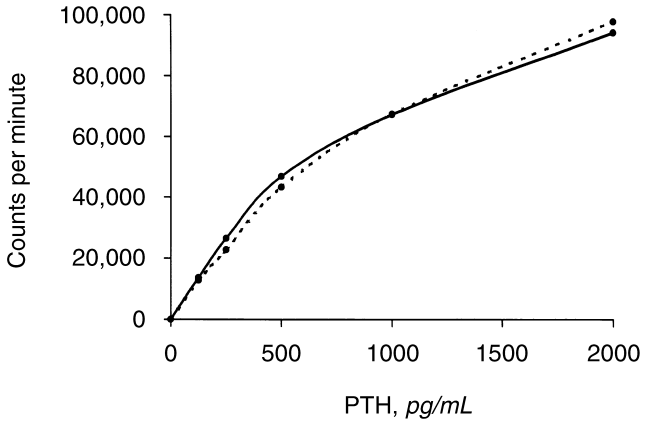

Fig. 2. Comparison of the recognition of hPTH 1-84 and hPTH 7-84 by the Nichols I-PTH assay. The Nichols I-PTH assay does not differentiate between hPTH 1-84 (solid line) and hPTH 7-84 (dashed line).

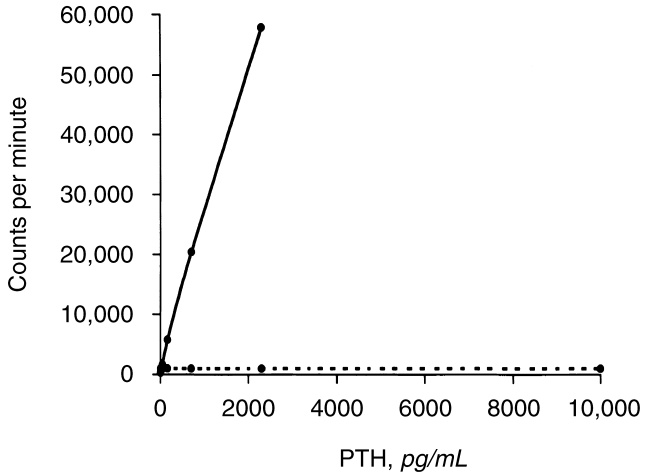

Fig. 3. Comparison of the recognition of hPTH 1-84 and hPTH 7-84 by the Whole PTH assay. Unlike the Nichols I-PTH assay, the Whole PTH assay does discriminate between hPTH 1-84 (solid line) and hPTH 7-84 (dashed line). Concentrations of hPTH 7-84 as high as 10,000 pg were undetectable.

Statistical analysis

Results are expressed as mean ± SEM. *N* indicates the number of samples. The paired *t*-test was employed to examine statistical significance, unless otherwise indicated in the text.

RESULTS

Specificity of IRMA assays for hPTH 1-84

Initial studies compared the ability of the Nichols Intact (I-Nichols) PTH assay and the new Whole PTH assay to discriminate between the hPTH 1-84 and hPTH 7-84 molecules. Figure 2 shows that the Nichols "intact" PTH assay did not discriminate between human PTH 1-84 and 7-84. However, as depicted in Figure 3, studies

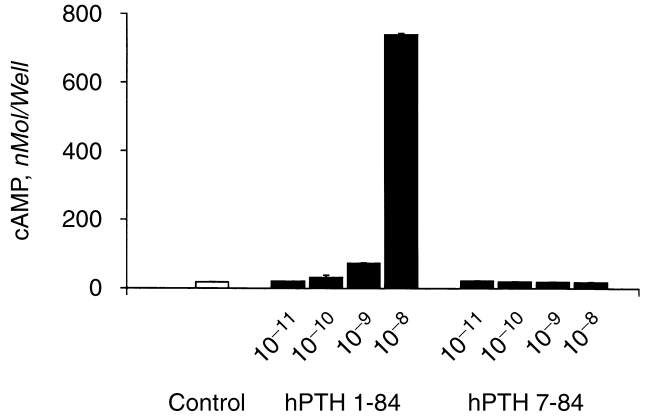

Fig. 4. Comparison of the effect of hPTH 1-84 or hPTH 7-84 on cAMP production in ROS 17.2 cells. Unlike hPTH 7-84, hPTH 1-84 increased cAMP production in a dose-dependent manner. cAMP increased from 18.1 ± 1.2 to 738 ± 4.1 nmol/well after treatment with 10^{-8} mol/L hPTH 1-84. The same concentration of hPTH 7-84 had no effect.

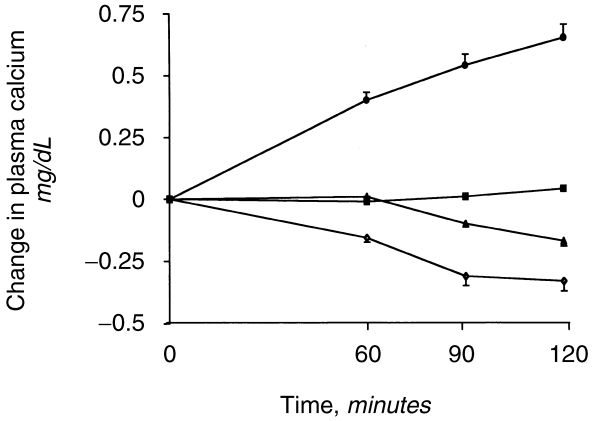

Fig. 5. Comparison of the calcemic effects of PTH isoforms. Parathyroidectomized (PTX) rats fed a 0.02% calcium diet show a significant increase in plasma calcium after treatment with hPTH 1-84. In contrast, hPTH 7-84 produced a slight but significant decrease in plasma calcium. When both peptides were given together in a 1:1 molar ratio, the calcemic response induced by hPTH 1-84 was reduced by 94% ($P < 0.001$). Symbols are: (●) 1-84, $N = 9$; (■) 1-84 + 7-84, $N = 6$; (▲) Control, $N = 5$; (◆) 7-84, $N = 5$.

performed using the Whole PTH assay show that hPTH 1-84 was detected with a high degree of sensitivity, whereas hPTH 7-84 was undetectable, even at a concentration as high as 10,000 pg/mL.

Studies in vitro

The results of cAMP production by ROS/17.2 cells exposed to hPTH 1-84 or hPTH 7-84 are shown in Figure 4. Unlike hPTH 7-84, hPTH 1-84 increased cAMP production in a dose-dependent manner. hPTH 1-84 (10^{-8} mol/L) increased intracellular cAMP from 18.1 ± 1.25 to 738 ± 4.13 nmol/well. On the other hand, the same concentration of hPTH 7-84 had no effect on cAMP ($N = 6$).

Studies in vivo in rats

We next examined the hPTH 7-84 fragment as a potential competitive inhibitor of hPTH 1-84 in bone by measuring changes in serum calcium in PTX rats. Figure 5 shows that the administration of hPTH 1-84 to PTX rats fed a 0.02% calcium diet increased plasma calcium by 0.65 ± 0.10 mg/dL ($N = 9$, $P < 0.001$, ANOVA). With the administration of vehicle alone, plasma calcium changed slightly in accordance with the PTX (−0.17 ± 0.10 mg/dL, $N = 5$). A slight but significant decrease was observed in the rats receiving hPTH 7-84 (−0.30 ± 0.08 mg/dL, $N = 5$, $P < 0.05$). When both peptides were given together in a 1:1 molar ratio, the calcemic response induced by the administration of hPTH 1-84 alone decreased by 94% ($N = 6$, $P < 0.001$, ANOVA). Thus, in this model, hPTH 7-84 significantly inhibits hPTH 1-84 induction of bone calcium mobilization.

The phosphaturic effects of these two peptides were then evaluated (Fig. 6). The GFR did not change in rats infused with hPTH 1-84 (1.8 ± 0.3 vs. 1.8 ± 0.1 mL/min), whereas fractional excretion of phosphate (FE_{PO4}) increased from 11.9 ± 2.4 to 27.7 ± 2.4% ($N = 10$, $P < 0.001$). When hPTH 7-84 was given simultaneously with hPTH 1-84, the GFR increased from 2.1 ± 0.1 to 2.6 ± 0.2 mL/min ($N = 8$, $P < 0.05$). However, despite this increase in GFR, the increase in FE_{PO4} induced by treatment with hPTH 1-84 was significantly decreased by 50.2% ($P < 0.01$) by virtue of the coadministration of PTH 7-84.

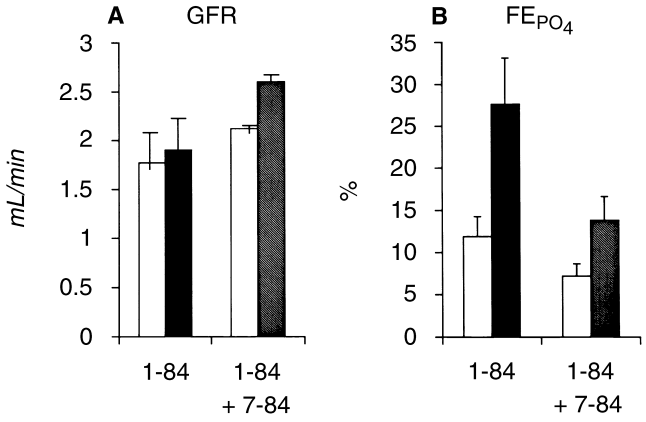

Fig. 6. Comparison of the effects of hPTH 1-84 or hPTH 1-84 plus hPTH 7-84 on (*A*) glomerular filtration rate (GFR) and (*B*) fractional excretion of phosphorus (FE_{PO4}). Control and treatment periods are denoted by open and closed bars, respectively. The phosphaturia induced by hPTH 1-84 was decreased by 50.2% ($P < 0.05$) when animals were treated simultaneously with 7-84 PTH, despite a significant increase in GFR ($P < 0.005$).

Studies in humans

Figure 7 shows that the values for plasma PTH were higher in all 28 patients on chronic dialysis when mea-

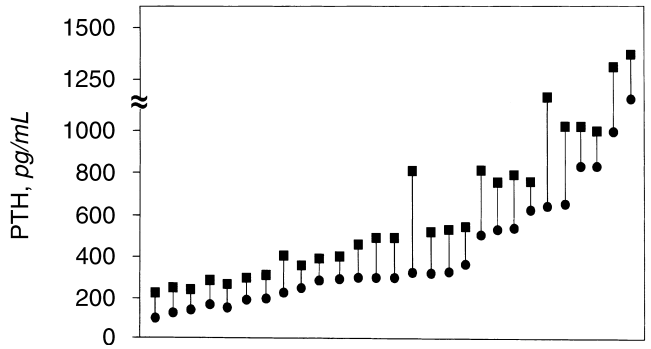

Fig. 7. Comparison of PTH values in plasma from uremic patients using the Nichols "intact" PTH assay (■) versus the Whole PTH assay (●). Plasma PTH values are uniformly higher when measured with the Nichols "intact" PTH assay than with the Whole PTH assay. The median PTH values were 523 vs. 344 pg/mL, respectively ($P < 0.001$).

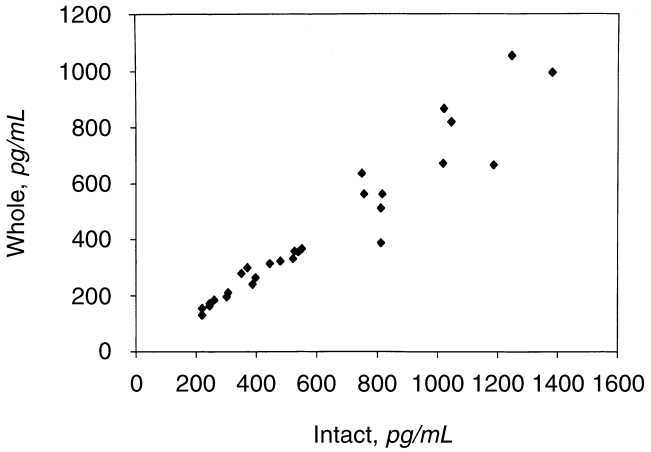

Fig. 8. Regression analysis of plasma PTH measured by Nichols I-PTH and Whole PTH assay in uremic patients ($r = 0.97$; $P < 0.001$).

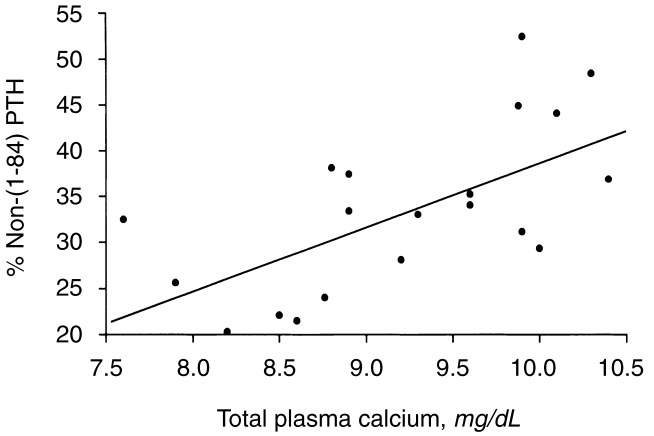

Fig. 9. Effects of plasma calcium on PTH degradation in dialysis patients. The percentage of non-(1-84) PTH fragment (likely hPTH 7-84) correlates positively with plasma calcium ($P < 0.02$) ($r = 0.638$; $P = 0.0025$; $N = 20$).

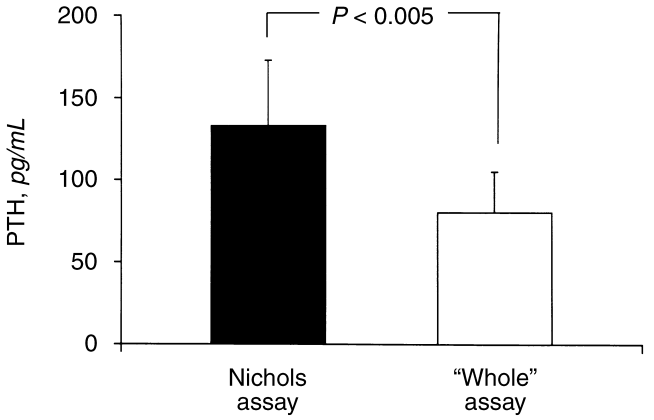

Fig. 10. Comparison of plasma PTH levels in renal transplant patients using Nichols I-PTH and Whole PTH assays. PTH values are higher when measured with the Nichols I-PTH assay ($P < 0.005$).

sured with the I-Nichols assay compared with the Whole assay. The median PTH values were 523 versus 344 pg/mL ($P < 0.001$), respectively. A regression analysis of these data is shown in Figure 8.

The association between plasma levels of non-(1-84) PTH, "likely" hPTH 7-84, and plasma calcium and phosphorus was next examined in 20 patients maintained on chronic dialysis (Fig. 9). There was a positive correlation between the percentage of non-(1-84) PTH and serum calcium ($P < 0.002$), but no correlation with plasma phosphorus (data not shown). These studies were performed only in those patients in whom there were values for calcium, phosphorous, and PTH from the same blood sample [20].

In a group of 14 renal transplant patients the percentage of non-(1-84) PTH was found to be 44.1 ± 3.1% of the total PTH, as measured by the I-Nichols assay and the Whole PTH assay (Fig. 10). The absolute PTH value with the I-Nichols assay was 132.9 ± 39.9 compared with 79.8 ± 24.8 pg/mL ($P < 0.005$) with the Whole PTH assay.

Finally, we examined whether intracellular cleavage of the hPTH 1-84 molecule occurs in the parathyroid gland, thus producing the non-(1-84) PTH fragment. Surgically excised parathyroid glands from six uremic patients maintained on chronic dialysis were studied. Figure 11 shows that non-(1-84) PTH fragments exist in the cell lysates from these parathyroid glands and represent 41.8 ± 3.2% ($P < 0.05$) of the total intracellular PTH measured by the "intact" PTH assay (that is, 1-84 PTH and most likely 7-84 PTH).

DISCUSSION

The results of this study clearly indicate that, in addition to the biologically active 1-84 PTH molecule, a trun-

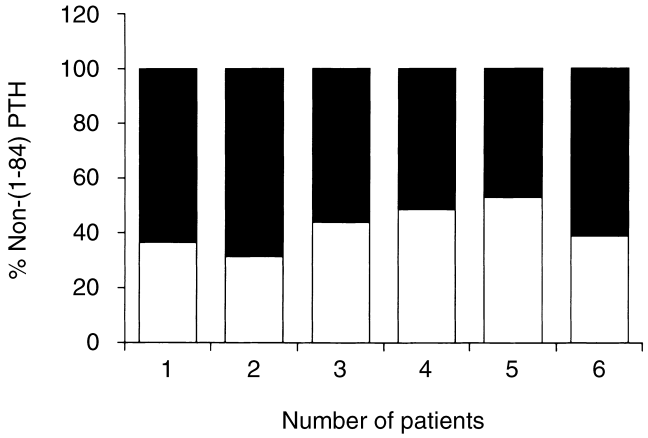

Fig. 11. Intracellular PTH content in parathyroid glands from uremic patients. The 41.8 ± 3.2% of the total PTH, measured by the I-Nichols assay (expressed as 100%), represents the non-(1-84) PTH fragment "likely" hPTH 7-84 (□). The 1-84 PTH molecule was measured with the Whole PTH assay (■).

cated non-(1-84) PTH fragment is also present in the blood and parathyroid cells of uremic patients. Although it has been accepted that commercial IRMA assays will recognize only the 1-84 PTH biologically active peptide, our data confirmed that the Nichols I-PTH assay also recognizes a truncated PTH fragment (7-84), which is biologically inactive. On the other hand, the new assay (Whole PTH assay) measures only the biologically active hPTH 1-84, since concentrations of hPTH 7-84 as high as 10,000 pg/mL were not recognized by this assay.

The specificity of the Whole PTH assay along with the significant concentrations and potent inhibitory activity of PTH 7-84 may have significant clinical implications. Quarles, Lobaugh, and Murphy clearly demonstrated with the Nichols "intact" PTH assay that higher concentrations of PTH, two to three times the levels found in normal patients, are usually associated with normal bone histology in uremic patients [22]. On the other hand, normal values of PTH obtained with the Nichols I-PTH assay are usually correlated with adynamic bone disease. Previously, other investigators also found skeletal resistance to PTH [28]. Thus, it has been a common practice among nephrologists to maintain the levels of I-PTH in the range of 200 to 300 pg/mL to prevent the development of adynamic bone disease.

We performed further studies with the human PTH 7-84 fragment and found that not only is it biologically inactive as far as the production of cAMP in vitro in ROS 17.2, but also has antagonistic effects against the biologically active 1-84 PTH when given to PTX rats. In these animals, we found an increase in serum calcium when biologically active PTH 1-84 was given alone. On the other hand, when both peptides were given in a molar ratio of 1:1, a significant suppression in the biological activity of hPTH 1-84 was seen. This decrease in the calcemic response was approximately 94%. Moreover, similar findings were seen at the renal level. The phosphaturia observed in rats after the administration of the biologically active 1-84 PTH molecule was decreased by 50.2% when both peptides were given together in a molar ratio of 4:1 (hPTH 7-84 to hPTH 1-84). Thus, not only at the level of the bone but at the level of the kidney, we have demonstrated that the 7-84 PTH fragment has antagonistic effects against the biologically active PTH 1-84.

In addition, we have analyzed samples from 28 patients maintained on chronic dialysis. As is shown in Figure 7, a significantly higher value was observed when PTH was measured by the Nichols I-PTH assay compared with the Whole PTH assay. The median PTH values were 523 versus 344 pg/mL, respectively ($P < 0.001$). Moreover, we have correlated the concentration of serum calcium with the percentage of the non–1-84 PTH fragment (likely hPTH 7-84), and found a positive correlation ($P < 0.002$; Fig. 9). In the presence of hypercalcemia, not only is PTH secretion suppressed but the parathyroid gland degrades a greater amount of biologically active PTH, forming fragments which are biologically inactive [7, 8]. In addition to the results in patients with end-stage renal disease, we also studied a group of 14 renal transplant patients. As seen in the end-stage renal disease patients, higher PTH values were obtained with the Nichols "intact" PTH assay versus the Whole PTH assay in every patient. Finally, we studied the origin of the non-(1-84) PTH fragment, and found that this fragment is made in the parathyroid gland itself. Both peptides were measured in lysates from the parathyroid glands of uremic patients using the two IRMA assay (Fig. 11). We found that 41.8 ± 5.2% of the total PTH measured in these lysates represents the non-(1-84) PTH fragment.

From a clinical point of view, the results from this study offer a possible explanation for the development of adynamic bone disease. When early attempts were made to suppress serum I-PTH to normal levels, many investigators found that, although osteitis fibrosa was eliminated, a new condition developed in these patients. This condition, adynamic bone disease, is characterized by a low number of osteoblasts and osteoclasts, a decreased amount of osteoid tissue, an absence of marrow fibrosis, and a mineralization defect. The development of adynamic bone disease may not be only a histologic finding. New evidence is emerging that this condition may produce morbidity in some patients [29]. Some patients have a propensity to develop hypercalcemia, which may result from impaired calcium flux into bone [30].

Our studies provide several clinically important findings. First, commercial I-PTH assays, specifically the Nichols I-PTH assay, does not measure only true biologically active 1-84 PTH, but in addition, measures biologi-

cally inactive peptide, likely 7-84 PTH. Similar results were recently reported by other investigators [abstracts; Brossard et al, *J Bone Miner Res* 14(Suppl 1):444, 1999, and Gao et al, *J Bone Miner Res* 14(Suppl 1):446, 1999] [31]. Second, the biologically inactive peptide, 7-84 PTH, has antagonistic effects in vivo in rats at the levels of bone and kidney. Third, significant differences in plasma PTH values were seen in uremic patients and those with renal transplants when using the two assays. Fourth, the origin of the non-(1-84) PTH truncated fragment is the parathyroid gland itself. Thus, the development of this new assay (Whole) opens a new approach to the study of metabolic bone disease and potential treatment in patients with renal failure. This assay measures only biologically active PTH and hopefully, in the future, will allow investigators to establish a better correlation between biologically active PTH and bone histology. It is not known at the present time if the Whole PTH assay is more sensitive in the diagnosis of primary hyperparathryoidism; therefore, further studies are mandatory to establish the clinical sensitivity of the two assays. Nevertheless, the fact that true biologically active PTH can now be specifically measured will allow investigators to further our knowledge of PTH secretion, metabolism, and its relationship to the development of adynamic bone disease.

In conclusion, the elevated presence of an antagonistic truncated PTH fragment of biologically active 1-84 PTH (likely 7-84 PTH) in the blood of uremic patients may potentially contribute to the higher levels of I-PTH necessary to prevent adynamic bone disease.

ACKNOWLEDGMENTS

This work was supported in part by U.S. Public Health Service NIADDA Grants AM-09976, AM-07126, and RR-00036. Scantibodies provided the "Whole" PTH kit used in these studies. P.G. and T.M. are members of Scantibodies Laboratory and have financial interest in this institution. The authors are grateful to Ms. Sue Viviano for assistance in the preparation of this manuscript.

Reprint requests Eduardo Slatopolsky, M.D., Washington University School of Medicine, Renal Division, 660 South Euclid Avenue, Box 8126, St. Louis, Missouri 63110, USA.

REFERENCES

1. Keutman HT, Sauer MM, Hendy GN, O'Riordan JL, Potts JT Jr: Complete amino acid sequence of human parathyroid hormone. *Biochemistry* 17:5723–5729, 1978
2. Habener JF, Potts JT Jr, Rich A: Pre-pro-parathyroid hormone: Evidence for an early biosynthetic precursor of pro-parathyroid hormone. *J Biol Chem* 251:3893–3901, 1976
3. Habener JF, Rosenblatt M, Kemper B, Kronenberg HM, Rich A, Potts JT Jr: Pre-pro-parathyroid hormone: Amino acid sequence, chemical synthesis and some biological studies of the precursor region. *Proc Natl Acad Sci USA* 75:2616–2621, 1978
4. Chu LLH, MacGregor RR, Hamilton JW, Cohn DV: Conversion of pro-parathyroid hormone to parathyroid hormone: The use of amines as specific inhibitors. *Endocrinology* 95:1431–1439, 1974
5. Sherwood LM, Mayer GP, Ramberg CF: Regulation of parathyroid hormone secretion: Proportional control by calcium: Lack of effect of phosphate. *Endocrinology* 83:1043–1051, 1968
6. Patt HM, Luckhardt AB: Relationship of low blood calcium to parathyroid secretion. *Endocrinology* 31:384–393, 1942
7. Chu LLH, MacGregor RR, Hamilton JW, Cohn DV: Studies on the biosynthesis of rat parathyroid hormone and pro-parathryoid hormone: Adaption of the parathyroid gland to dietary restriction of calcium. *Endocrinology* 93:915–924, 1973
8. Habener JF, Kemper B, Potts JT Jr: Calcium dependent intracellular degradation of parathyroid hormone: A possible mechanism for the regulation of hormone stores. *Endocrinology* 97:431–441, 1975
9. Coburn JW, Slatopolsky E: Vitamin D, parathyroid hormone, and the renal osteodystrophies, in *The Kidney*, edited by Brenner B, Rector F, Philadelphia, W.B. Saunders, Co., 1991, pp 2036–2120
10. Coburn JW, Salusky I: Hyperparathyroidism in renal failure: Clinical features, diagnosis, and management, in *The Parathyroid Basic and Clinical Concepts*, edited by Bilizikian JP, Levine MA, Marcus R, New York, Raven Press, 1994, pp 721–745
11. Nussbaum SR, Zahardiuk RJ, Lavigne JR, Brennan GL, Nozawa-Ung K, Kim LY, Keutman HT, Wang C, Potts JT Jr: Highly sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. *Clin Chem* 88:1364–1371, 1987
12. Brown PC, Aston JP, Weeks I, Woodheard JS: Circulating intact parathyroid hormone measured by a two-site immunochemiluminometric assay. *J Clin Endocrinol Metab* 65:407–412, 1987
13. Elkins R: Towards immunoassays of greater sensitivity, specificity and speed: An overview. in *Monoclonal Antibodies and Developments in Immunoassay*, edited by Albertine A, Elkins R, Amsterdam, Elsevier, North Holland, 1981, pp 3–21
14. Nussbaum SR, Potts JT Jr: Immunoassays for parathyroid hormone 1-84 in the diagnosis of hyperparathryoidism. *J Bone Miner Res* 6:S43–S50, 1991
15. Kao PC, Vanhererden JA, Grant CS, Klee GG, Khosla S: Clinical performance of parathyroid hormone immunometric assays. *Mayo Clinic Proc* 67:637–645, 1992
16. Hruska K, Kopelman R, Rutherford WE, Klahr S, Slatopolsky E: Metabolism of immunoreactive parathyroid hormone in the dog: The role of the kidney and the effects of chronic renal disease. *J Clin Invest* 56:39–48, 1975
17. Freitag J, Martin KJ, Hruska KA, Anderson C, Conrades M, Ladenson J, Klahr S, Slatopolsky E: Impaired parathyroid hormone metabolism in patients with chronic renal failure. *N Engl J Med* 298:29–32, 1978
18. Martin KJ, Hruska KA, Freitag JJ, Klahr S, Slatopolsky E: The peripheral metabolism of parathyroid hormone. *N Engl J Med* 301:1092–1098, 1979
19. Segre GV, D'Amour P, Hultman A, Potts JT Jr: Effects of hepatectomy, nephrectomy, and nephrectomy/uremia on the metabolism of parathyroid hormone in the rats. *J Clin Invest* 67:439–448, 1981
20. D'Amour P, Lazure C, Labelle F: Metabolism of radioiodinated carboxy terminal fragments of bovine parathyroid hormone in normal and anephric rats. *Endocrinology* 117:127–134, 1985
21. Mallette LC, Tuma SN, Berger RE, Kirkland J: Radioimmunoassay of the middle region of human parathyroid hormone using an homologous antiserum with carboxyl-terminal fragment of bovine parathyroid hormone as radio/ligand. *J Clin Endocrinol Metab* 54:1017–1024, 1982
22. Quarles LD, Lobaugh B, Murphy G: Intact parathyroid hormone overestimates the presence and severity of parathyroid mediated osseous abnormalities in uremia. *J Clin Endocrinol Metab* 75:145–150, 1992
23. Wang M, Hercz G, Sherrard DJ, Maloney NA, Segre GV, Pei Y: Relationship between 1 and 84 parathyroid hormone and bone histomorphometric parameters in dialysis patients without aluminum toxicity. *Am J Kidney Dis* 26:836–844, 1995
24. Lepage R, Roy L, Brossard JH, Rousseau L, Dorais C, Lazure C, D'Amour P: A non-(1-84) circulating parathyroid hormone fragment interferes significantly with intact PTH commercial assay measurement in uremic samples. *Clin Chem* 44:805–809, 1998
25. Brossard JH, Whittnom S, Lepage R, Gascom-Barre M,

D'Amour P: Carboxyl-terminal fragments of parathyroid hormone are not secreted preferentially in primary hyperparathyroidisim as they are in other causes of hypercalcemia. *J Clin Endocrinol Metab* 77:413–419, 1993

26. Steiner AL, Kipnis R, Uttiger R, Parker C: Radioimmunoassay for the measurement of adenosine 3′-5′ cyclic phosphate. *Proc Natl Acad Sci USA* 64:367–375, 1969
27. Führ JJ, Kaczmarczyk J, Kruttgen CD: Eine einfache colorimetrische methode zur inulinbestimmung fur nieren-clearance-untersuchungen bei stoffwechselgesunden und diabetikern. *Klin Wochenschr* 33:729–730, 1955
28. Llach F, Massry SC, Singer FR, Kurokawa K, Kaye JH, Coburn JW: Skeletal resistance to endogenous parathyroid hormone in patients with early renal failure, a possible cause for secondary hyperparathyroidism. *J Endocrinol Metab* 41:339–345, 1975
29. Chan YL, Furlong TJ, Cornish CJ, Posen S: Dialysis osteodystrophy: A study involving 94 patients. *Medicine (Baltimore)* 64:296–309, 1985
30. Kurz P, Monier-Faugere MC, Bognar C, Roth P, Vlachojamanis J, Malluche HH: Evidence for abnormal calcium homeostasis in patients with adynamic bone disease. *Kidney Int* 46:855–861, 1994
31. John MR, Goodman WG, Gao P, Cantor TL, Salusky IB, Juppner H: A novel immunoradiometric assay detects full-length human PTH but not amino-terminally truncated fragments: Implications for PTH measurements in renal failure. *J Clin Endocrinol Metab* 84:4287–4290, 1999