

Immunoheterogeneity of Parathyroid Hormone in Venous Effluent Serum from Hyperfunctioning Parathyroid Glands

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ABSTRACT The immunoreactive parathyroid hormone (iPTH) in the plasma of hyperparathyroid man consists largely of carboxyl (COOH)-terminal fragments of the hormone. Although these fragments have been thought to arise principally or solely from peripheral metabolism of intact human PTH {hPTH(1-84)} secreted from the parathyroid gland, there is disagreement about the source of iPTH fragments in vivo.

To reexamine this question, we fractionated peripheral and thyroid or parathyroid venous effluent sera from four patients with primary hyperparathyroidism using a high-resolution gel filtration system (Bio-Gel P-150 columns run by reverse flow). The column effluents were analyzed using two PTH radioimmunoassays, one directed toward the amino(NH₂)-terminal region of the molecule, the other toward the COOH-terminal region.

In all four thyroid or parathyroid venous effluent sera studied, iPTH was 9–180 times higher than in peripheral serum from the same patient; after fractionation, hPTH(1-84) accounted for only a portion of the total iPTH (35–55% with the assay directed toward the COOH-terminal region of hPTH, >90% with the NH₂-terminal directed assay.) The remaining iPTH

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eluted from Bio-Gel P-150 after hPTH(1-84) as NH₂- or COOH-terminal hPTH fragments. These results suggest that parathyroid tumors secrete large quantities of hPTH fragments. Based on estimates of their molar concentrations in serum, tumor-secreted COOH-terminal hPTH fragments could account for most of these peptides in peripheral serum if their survival times were, as estimated by several other workers, 5–10 times that of hPTH(1-84).

We conclude that, in contrast to published information, secretory products of hyperfunctioning parathyroid tissue are probably a major source of serum PTH immunoheterogeneity.

INTRODUCTION

As is the case for several other peptide hormones (1), there has been keen interest in the molecular forms of parathyroid hormone (PTH)¹ and its fragments that circulate in human blood. In 1968, Berson and Yalow made the novel observation that PTH in the plasma of patients with hyperparathyroidism is immunoheterogeneous (2), and several laboratories have subsequently confirmed and expanded on their findings (3–6). Recently, it has been shown that the immunoreactive PTH (iPTH) in hyperparathyroid plasma consists principally of at least two hormonal forms: intact PTH {PTH(1-84)} and carboxyl (COOH)-terminal fragments of PTH(1-84) (7–13). There is also general agreement that the concentration of COOH-

¹Abbreviations used in this paper: COOH- and NH₂-terminal, carboxyl- and amino-terminal respectively; iPTH, immunoreactive PTH; PTH, parathyroid hormone; PTH(1-84), intact PTH, peptide of 84 amino acids; PTH(1-34), synthetic amino-terminal peptide of PTH, peptide of 34 amino acids.

terminal PTH fragments in such plasma is 5–10 times higher than that of PTH(1-84); this concentration difference is thought to be due to the longer survival time of these COOH-terminal fragments in plasma (5, 8, 14).

The important question of where these PTH fragments originate is unsettled. Two alternatives have been proposed. The first is that PTH fragments are derived almost exclusively from peripheral metabolism of secreted hPTH(1-84) (15). Potts et al. (6, 9, 15–19) have reported that virtually all of the iPTH in venous effluent blood from parathyroid adenomas coeluted with ¹³¹I-bovine PTH [bPTH(1-84)] after gel filtration on columns of Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.). These researchers have also demonstrated specific and sensitive cleavage of exogenously administered PTH(1-84) and labeled PTH(1-84) in several animal species (9, 20, 21). The second alternative is that PTH fragments are directly secreted from parathyroid tissue; before information was available concerning the molecular forms of iPTH in plasma, Arnaud et al. (3, 4), and Sherwood et al. (22) showed that PTH fragments were released into the medium by parathyroid adenoma explants cultured in vitro.

Recently, Silverman and Yalow have critically reviewed published information concerning the potential sources of circulating COOH-terminal fragments, in the course of their own studies of PTH immunoheterogeneity in parathyroid tumor extracts and hyperparathyroid plasma (8). These workers pointed out the significance of the much longer half-life of COOH-terminal PTH fragments relative to that of PTH(1-84) in any consideration of the source of these hormonal forms in plasma. It was suggested that only a relatively small quantity of COOH-terminal frag-

ments, which might not have been adequately resolved from PTH(1-84) by the gel-filtration experiments of Potts et al. (6, 9, 15–19), needed to be released by parathyroid tissue to account for their high concentrations in peripheral plasma.

In the studies to be described here, we have re-examined the question regarding the origin of COOH-terminal PTH fragments. We have carried out gel-filtration studies on peripheral and parathyroid venous effluent sera from four patients with primary hyperparathyroidism using a high-resolution gel filtration system, especially developed to resolve PTH(1-84) from its fragments. In contrast to other reports (9, 12, 16–19) we have been able to demonstrate that at least one-third of the total iPTH in the parathyroid venous effluents from these four patients consists of COOH-terminal fragments of PTH(1-84). We also find that, assuming the half-life in plasma of COOH-terminal PTH fragments is 5–10 times longer than that of hPTH(1-84) (5, 8, 14), these gland-derived PTH fragments could account for a large portion (20–100%) of the COOH-terminal PTH fragments in the peripheral sera of these hypercalcemic patients.

METHODS

Patients. Four female patients with primary hyperparathyroidism were studied before (during catheterization procedures) or at the time of surgical removal of hyperfunctioning parathyroid tissue. Clinical data (Table I) indicated hypercalcemia with inappropriately high levels of serum iPTH (23, 24); renal function was normal or only minimally impaired for the patients' ages (25). Pathological examination of tissue removed at surgery showed the characteristics of parathyroid chief cell adenoma in two patients and chief cell hyperplasia in the other two.

Serum samples. Two sets of blood samples (from patients

TABLE I
Clinical Data on Four Patients with Primary Hyperparathyroidism

| Patient | Pathologic diagnosis | Age | Serum values | | | | Creatinine clearance |
|--------------|----------------------|-----------|--------------|--------------|-----------------|----------------|----------------------------------|
| | | | Calcium | Phosphate | iPTH* | Creatinine | |
| | | <i>yr</i> | <i>mg/dl</i> | <i>mg/dl</i> | <i>μl eq/ml</i> | | <i>ml/min/1.73 m²</i> |
| I | Hyperplasia‡ | 67 | 10.4 | 2.7 | 510 | 1.7 | 51 |
| II | Adenoma§ | 63 | 12.4 | 2.4 | 240 | 1.6 | 50 |
| III | Adenoma | 61 | 13.6 | 1.9 | 770 | 1.0 | 66 |
| IV | Hyperplasia | 22 | 11.3 | 2.8 | 150 | 0.8 | 106 |
| Normal range | | | 8.9–10.1 | 2.5–4.5 | <40 | Reference (25) | |

* Expressed in terms of microliters equivalents standard hyperparathyroid serum per milliliter (24). Serum samples for clinical data above were not necessarily obtained at the same time as those used in the simultaneous comparison of iPTH in peripheral and parathyroid venous effluent sera. Antiserum used was GPIM (Anti-C).

‡ Multiple gland involvement.

§ Single gland involvement.

II and III) were collected at surgery. After identification of an adenoma in patient II, a small polyethylene catheter was inserted into the right inferior thyroid vein and advanced to a position just distal to the origin of the parathyroid vein, where the sample was allowed to flow into a small test tube. In patient III, a large extracapsular vein directly draining a 17-g adenoma was carefully incised cleanly without touching parathyroid tissue. The blood sample was collected dropwise in a small beaker placed below the severed vein. Two additional blood samples were obtained during venous catheterization for localization of hyperplastic tissue. Samples from patient I were collected from the right superior thyroid vein. Samples from patient IV were obtained after arteriographic localization of an aberrantly located fifth parathyroid gland. Several days later, the vein draining only this gland (macroscopically confirmed at surgery) was catheterized for confirmation of a venous gradient. Thus, two patients (III and IV) had samples collected from parathyroid veins, whereas the other two (I and II) were from thyroid veins. Peripheral blood was obtained without stasis from a superficial arm vein at the time of procurement of thyroid or parathyroid venous blood. In all instances, blood was allowed to clot at 22°C for 1 h and then centrifuged at 4°C; serum was removed (the buffy coat was excluded), and frozen at -20°C within 2 h of procurement. One of the blood specimens obtained from a thyroid vein (II, Table II) was collected in the presence of 2,000 kallikrein inactivator units of the proteolytic enzyme inhibitor, Trasylol (FBA Pharmaceuticals Inc., New York) per 3 ml. Peripheral serum from a surgically hypoparathyroid patient who had undetectable concentrations of iPTH (GP1M antiserum) was used in the control gel-filtration experiments (see section on gel-filtration procedures).

Radioimmunoassays and specificity of antisera. The methods used have been described in detail (24) and were employed with minor modifications for all radioimmunoassays of PTH done on whole serum and on gel-filtration column-effluent fractions. In brief, our procedures involved the preincubation of antiserum, standard or unknown in 300 μ l of assay diluent (0.1 M sodium barbital buffer, pH 8.6, containing 10% hypoparathyroid plasma and 500 kallikrein inactivator units Trasylol/ml) for 3 days, shaking at 4°C, followed by 2 more days of incubation with ¹³¹I-bPTH(1-84) added in 200 μ l of assay diluent (total incubation volume, 500 μ l). Separation of antibody-bound from "free" (B/F) tracer was carried out with dextran-coated charcoal by a modification (24) of the procedure of Herbert et al. (26). The quality control measures reported (24) were also used in the present studies. In particular, for a valid iPTH measurement we required that: (a) addition of unknown serum specimens or column fractions produced decreases in the B/F ratio at least 2 SD greater than the mean B/F ratios obtained with the same quantities of hypoparathyroid serum or column buffer processed in the same way as column fractions containing iPTH; and (b) iPTH values obtained from measurements of at least two but usually three or more dilutions of the same unknown serum or column fractions were within $\pm 10\%$ of each other.

bPTH(1-84) isolated by described procedures (27, 28) was labeled with ¹³¹I (29) and used as the tracer in all radioimmunoassays. Homogeneous hPTH(1-84) (30) was used as standard. In the radioimmunoassays with antiserum GP1M the standard curve produced by multiple dilutions of hyperparathyroid serum was parallel to the curve produced by hPTH(1-84) over its initial two-thirds, however, the curves diverged at higher concentrations (11). Only the initial two-thirds of the hPTH(1-84) standard curve was therefore used to determine iPTH values with this antiserum. With antiserum

CH14M, however, the immunodilution curves of hyperparathyroid serum and hPTH(1-84) were completely parallel (11).

PTH was measured with two radioimmunoassay systems which have markedly different specificities for regions of the PTH molecule (11). One, with GP1M antiserum at a final dilution of 1:75,000, is directed primarily toward the COOH-terminal region of PTH and is therefore designated "anti-C". In this system, as little as 10 pg of hPTH(1-84) competitively inhibits the binding of ¹³¹I-bPTH(1-84), but no inhibition of this binding is observed with 10,000-fold (100 ng) greater quantities of either of the synthetic amino(NH₂)-terminal peptides bPTH(1-34) (31) or hPTH(1-34).² The other assay system is primarily directed toward the NH₂-terminal region of PTH. It uses CH14M antiserum at a final dilution of 1:2,000 and is designated "anti-N". In contrast to the anti-C system, the anti-N assay has virtually equivalent recognition for hPTH(1-84) and the synthetic NH₂-terminal peptides, bPTH(1-34), and hPTH(1-34) (11).

It should be stated, however, that definitive characterization of these antisera with respect to their ability or inability to recognize the COOH-terminal region of human PTH depends upon the results of studies with pure COOH-terminal fragments of hPTH. These peptide fragments are currently not available, and to the authors' knowledge, no reported PTH radioimmunoassay system has been characterized in this way. Therefore, we emphasize that our operational assumptions regarding the specificity characteristics of the two assay systems, used in the present studies, are valid only within the limits of the specificity studies we have done with human intact PTH obtained from natural sources and its synthetic amino-terminal fragment, hPTH(1-34).

Gel filtration. The methods used (10) have been modified for these studies. Columns (2.6 \times 90 cm, Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N.J.) of Bio-Gel P-150 (100-200 mesh; Bio-Rad Laboratories) were developed by reverse (ascending) flow at 4°C in 0.2 M ammonium-acetate buffer (pH 4.6). A constant flow rate of 6.5 ml/h was maintained using a Polystaltic Pump (Buchler Instruments Div., Searle Analytic Inc., Fort Lee, N. J.) and fractions were collected in 4.5- to 4.9-ml volumes in 25 \times 80-mm flint glass vials (Amersham/Searle Corp., Arlington Heights, Ill.).

Serum samples to be gel filtered were thawed after storage for no longer than 6 mo, brought to a temperature of 4°C, and centrifuged at 10,000 \times g for 15 min at 4°C. Each patient's peripheral, parathyroid or thyroid venous serum samples were applied separately to columns in the same volume (1-2 ml). In a separate experiment designed to determine if blood was capable of degrading PTH, 100 ng of homogeneous hPTH(1-84) was incubated in 5 ml of normal whole blood at 22°C for 1 h. The serum was removed after centrifugation, frozen at -20°C for 2 wk, and finally thawed, centrifuged, and gel filtered (1 ml) with the same procedures as described for hyperparathyroid sera. Immediately before application to columns, tracer amounts ($\approx 20,000$ cpm) of ¹³¹I-bPTH(1-84), ¹²⁵I-bPTH(1-34), and ¹²⁵I were added to each serum sample as column markers. Individual column fractions contained quantities of radioactivity which were well below levels that might have interfered with radioimmunoassays (<100 pg iPTH). The void volume (V₀) was indicated by the elution position of the largest serum protein component as determined by optical density determina-

² The amino acid sequence of the synthetic hPTH(1-34) (32) used in these studies was that reported by Brewer et al. (33, 34). A somewhat different amino acid sequence of hPTH(1-34) has been independently determined by others (35, 36).

tions at 280 nm, and the salt volume (V_s) was determined by the elution position of ^{125}I . K_d of the components in column effluents was calculated by the following formula:

$$K_d = (\text{elution volume of substance} - V_0) / (V_s - V_0).$$

Fig. 1A shows a representative gel-filtration profile using this fractionation system for separation of the calibration mixture of ^{131}I -bPTH(1-84), ^{125}I -bPTH(1-34), and ^{125}I which were added to 10 ml of hypoparathyroid plasma before

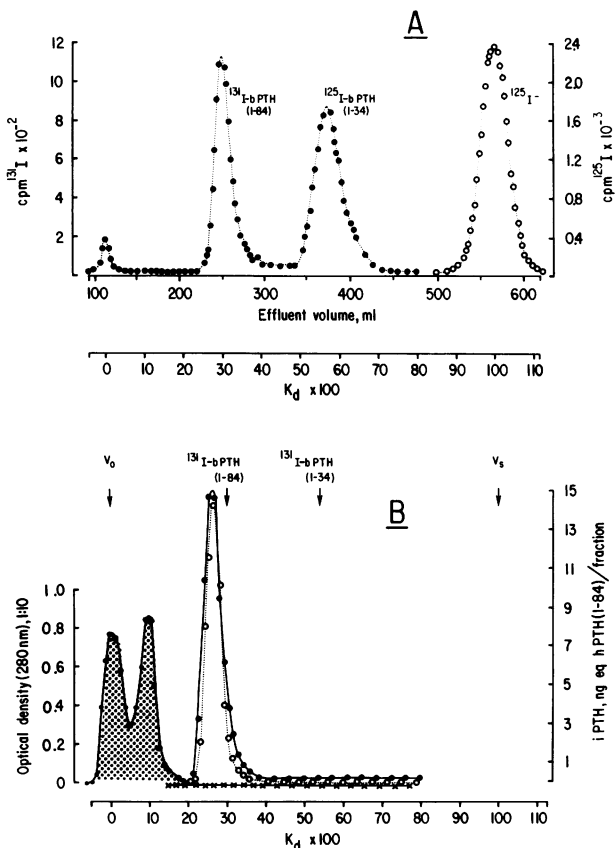


FIGURE 1 Resolution characteristics of Bio-Gel P-150 gel filtration system. (A) Pattern of radioactivity in column fractions after chromatography of 10 ml hypoparathyroid serum to which had been added $\approx 200,000$ cpm ^{131}I -bPTH(1-84), 200,000 cpm ^{125}I -bPTH(1-34), and 300,000 cpm ^{125}I . Details of chromatography and for calculation of K_d values are in text. (B) Gel filtration of a 1-ml sample of serum from normal whole blood to which 100 ng homogeneous hPTH(1-84) had been added (details of incubation in Methods); column fractions were analyzed for iPTH after lyophilization (see text for details) using the anti-N assay (CH14 antiserum, \circ) and the anti-C assay (GP1M antiserum, \bullet). Assay standard, homogeneous hPTH(1-84). Recovery of iPTH (both assays) was $>75\%$. Shaded area represents the optical density readings at 280 nm of 1:10 dilutions of the column fractions. (\times), iPTH (anti-N and anti-C assays) in lyophilized column fractions after gel filtration of 10 ml serum from a patient with post-surgical hypoparathyroidism. The detection limit for iPTH in all assays was ≈ 0.1 ng eq hPTH(1-84)/fraction. The void volume (V_0), the salt volume (V_s), and the elution positions of radioactive internal markers are indicated by the arrows.

chromatography. The resolution of these marker components is excellent: ^{131}I -bPTH(1-84) ($K_d = 0.297$) is separated by about 50 ml from the elution position of the most retained serum protein component and ^{131}I -bPTH(1-34) ($K_d = 0.565$) elutes about 180 ml before the salt volume. For nine column runs the mean K_d (\pm SD) for the ^{131}I -bPTH(1-84) markers was 0.301 ± 0.005 and for the ^{131}I -bPTH(1-34) marker was 0.556 ± 0.021 . Fig. 1B shows that homogeneous hPTH(1-84) preincubated in whole blood as described elutes as a single immunoreactive component from these columns ≈ 15 ml before the ^{131}I -bPTH(1-84) marker; the anti-N and anti-C assays measured hPTH(1-84) identically in effluent fractions processed as described below. These results indicate that detectable quantities of hPTH fragments are not generated from hPTH(1-84) under the conditions we used for processing blood and serum. No iPTH could be detected in column fractions when 10 ml of serum from a surgically hypoparathyroid patient was gel filtered and lyophilized as described below.

Systematic processing of effluent fractions included: (a) assay of radioactivity by gamma scintillation counting (2-inch well, Auto-Gamma Instrument, Searle Analytic Inc., Des Plaines, Ill.) at 22°C (total counting time 1.5 h); (b) optical density measurements at 280 nm of those column fractions containing serum proteins; (c) individual shell freezing of fractions; (d) lyophilization twice to remove ammonium acetate; (e) storage of the dried fractions at -20°C ; (f) solvation with 1 ml of assay diluent (refer to section on radioimmunoassay procedures); and (g) radioimmunoassay. Steps a-f were carried out in the same vials in which column fractions had been collected. Equal volumes of column buffer which had passed through gel-filtration columns before elution of serum proteins were processed in the same way as column fractions containing iPTH; the twice lyophilized residues were analyzed as radioimmunoassay "blanks" or used in control incubations for samples containing iPTH.

RESULTS

Assays of unfractionated hyperparathyroid sera (Table II). All values for serum iPTH were consistently greater when measured with the anti-C assay (GP1M antiserum) than with the anti-N assay (CH14M antiserum). The difference between iPTH values determined by the two assays was statistically significant ($P < 0.014$ by rank-sum test [37]) only in peripheral sera, however. In these sera, iPTH averaged 7.2 ng eq hPTH(1-84)/ml when determined with the anti-C assay and 1.6 ng eq hPTH(1-84)/ml when determined with the anti-N assay.

In thyroid or parathyroid venous effluent sera, we measured consistently greater quantities of iPTH with the anti-C assay (mean, 203 ng eq hPTH(1-84)/ml) than with the anti-N assay (mean, 143 ng eq hPTH(1-84)/ml). However, this difference was not statistically significant, probably because of the marked variation in total serum iPTH values from one patient to another. On the other hand, the ratio of anti-C iPTH to anti-N iPTH in these sera (mean \pm SD, 1.4 ± 0.1 ng hPTH(1-84)/ml) was significantly greater ($P < 0.001$) than the ratio of 1.0 observed when iPTH was measured in hypoparathyroid serum to which homogeneous

TABLE II
Concentrations of iPTH in Peripheral Sera and Thyroid Venous Effluent or Parathyroid Venous Effluent Sera from Four Patients with Surgically Proven Primary Hyperparathyroidism

| Patient | Sample | iPTH* | | iPTH(Anti-C) iPTH(Anti-N) |
|---------|----------------------------------------|--------------|--------|------------------------------|
| | | Anti-C | Anti-N | |
| | | <i>ng/ml</i> | | |
| I | Thyroid venous effluent | 106 | 69 | 1.5 |
| | Peripheral serum | 11.4 | 1.8 | 6.3 |
| | Step-up, increases‡ | 9.3 | 38 | — |
| II | Thyroid venous effluent | 380 | 274 | 1.4 |
| | Peripheral serum | 5.6 | 1.5 | 3.7 |
| | Step-up, increases | 68 | 183 | — |
| III | Parathyroid venous effluent | 150 | 93 | 1.6 |
| | Peripheral serum | 8.2 | 1.6 | 5.1 |
| | Step-up, increases | 18 | 58 | — |
| IV | Parathyroid venous effluent | 176 | 136 | 1.3 |
| | Peripheral serum | 3.6 | 1.5 | 2.4 |
| | Step-up, increases | 49 | 91 | — |
| Mean | Thyroid or parathyroid venous effluent | 203 | 143 | P NS |
| | Peripheral serum | 7.2 | 1.6 | <0.014 |
| | Step-up, increases | 28 | 89 | — |

* Determined by two radioimmunoassays, using antisera that recognize the amino-terminal (anti-N assay: antiserum CH14M) or carboxyl-terminal (anti-C assay: antiserum GP1M) region of hPTH. Assay standard, homogeneous hPTH(1-84). See text for details.

‡ Given as the ratio of thyroid vein or parathyroid vein iPTH/peripheral iPTH.

§ Significantly greater than the ratio of 1.0 observed when iPTH was measured in hypoparathyroid serum ($P < 0.001$) (37) to which highly purified hPTH(1-84) had been added.

^{||} For the difference between the iPTH values determined by anti-N and anti-C assays; the rank-sum test was used for statistical analysis (37).

hPTH(1-84) had been added. These observations suggested that COOH-terminal fragments of hPTH were present in thyroid or parathyroid venous effluents in addition to intact hormone.

A 9.3-fold or greater "step-up" increase was observed in all four thyroid or parathyroid venous effluent sera when compared to their respective peripheral sera (range, 9.3- to 183-fold). These large step-up increases indicate that the iPTH which we measured in thyroid or parathyroid venous effluent was derived largely from parathyroid tissue. Step-up increases were consistently greater when iPTH was measured with the anti-N assay (range, 38- to 183-fold; mean, 89-fold) than with the anti-C assay (range, 9.3- to 68-fold; mean, 23-fold). However, this difference was not due, as suggested (38), to relatively greater quantities of iPTH in thyroid or parathyroid venous effluents when measured with the anti-N assay. There was actually less iPTH in these sera. The greater step-up difference observed with the anti-N assay resulted from the much lower quantities of iPTH in peripheral sera measured with this assay than measured with the anti-C assay.

Serum fractionation on Bio-Gel P-150 columns. Gel-filtration patterns of iPTH (anti-C assay only) in the four thyroid or parathyroid venous effluent sera studies are shown in Fig. 2A-D. The corresponding pattern for iPTH in an equal volume of peripheral serum from each patient, shown for comparison, confirms that iPTH in peripheral serum made only a small contribution (<10% in all cases) to the total iPTH observed in the patient's thyroid or parathyroid venous effluent serum. We consistently observed a large peak of immunoreactivity in all four thyroid or parathyroid venous effluent sera that eluted just before the ¹³¹I-bPTH(1-84) marker. Mean K_d (\pm SD) for this component in four column runs was 0.271 ± 0.007 , and it reacted equally well in both anti-C and anti-N assays (Fig. 3A). We interpreted these observations to indicate that this peak corresponded to hPTH(1-84).

In addition to intact hPTH, however, we consistently observed large quantities of iPTH in all four thyroid or parathyroid venous effluent sera studied that eluted after hPTH(1-84) but well before the salt volume; the K_d for these iPTH components ranged from ≈ 0.35

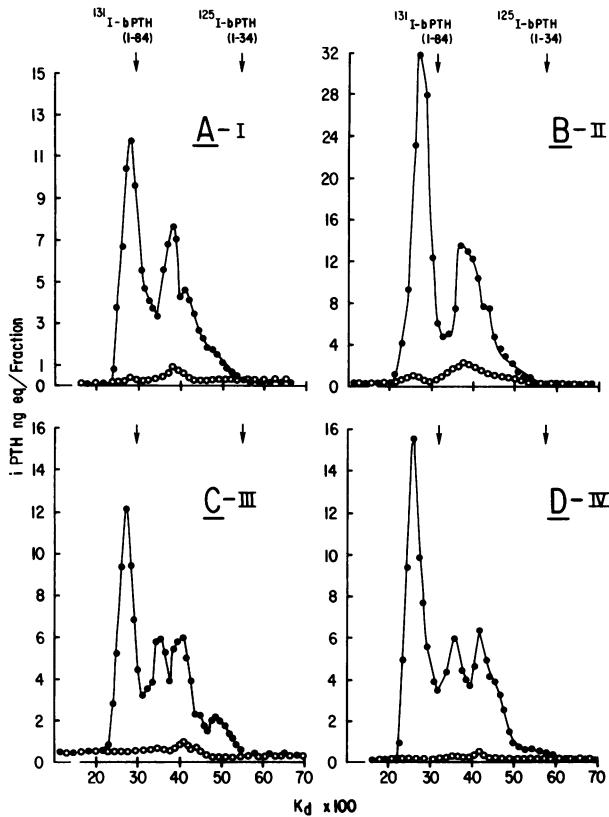


FIGURE 2 Elution patterns of iPTH (anti-C assay only) after gel filtration of serum samples from patients I-IV (Tables I and II). For simplicity, the regions of the elution profiles representing the optical densities (at 280 nm) of the serum proteins are not shown. Details of the gel filtration and processing of the fractions are in the text. Column fractions (after lyophilization) were analyzed for iPTH with the anti-C assay (GP1M antiserum). (●), sera obtained from thyroid (A and B) or parathyroid (C and D) veins; (○), peripheral serum. The volumes of thyroid or parathyroid and peripheral serum gel filtered for each individual patient were the same: A, 1.8 ml; B, 1.0 ml; C, 2.0 ml; D, 1.0 ml. The arrows show the elution positions of radioactive internal markers. The detection limit for iPTH in all assays was ≈ 0.1 ng eq hPTH (1-84)/fraction. Recoveries of iPTH ranged from ≈ 50 to 70% and were not significantly different between thyroid or parathyroid and peripheral sera.

to 0.6. Integration of these four gel-filtration profiles showed that ≈ 30 –50% of the total iPTH in thyroid or parathyroid venous effluent sera consists of these immunoreactive PTH fragments.

Rechromatography of the iPTH component in parathyroid venous effluent serum with a K_d of 0.27 using the same column system again showed a single symmetrical peak of immunoreactivity that was equally reactive in anti-N and anti-C assays; there was no detectable iPTH trailing from this peak (data not shown). Rechromatography of the pooled components in parathyroid venous effluent serum eluting with K_d of

≈ 0.35 –0.6 gave only a series of iPTH peaks with these same K_d values; no immunoreactivity could be detected in the region of the elution pattern corresponding to hPTH(1-84) (data not shown).

Examination (anti-N and anti-C assays) of the iPTH in thyroid venous effluent serum from patient I after gel filtration (Fig. 3A) showed that $\approx 90\%$ of the iPTH detected in the anti-N assay (determined by integration) consisted of hPTH(1-84). The remainder consisted of an iPTH component with K_d of 0.4–0.5 which reacted predominantly in the anti-N assay. We interpreted these observations to indicate the presence of an NH_2 -terminal PTH fragment. Three of the four thyroid or parathyroid venous effluent sera (I, II, and IV) contained this iPTH fragment (data not shown), and it comprised 5–10% of the total iPTH measured with the anti-N assay in these three sera. Fig. 3B (note different scale) shows the gel-filtration profile of iPTH in peripheral serum from Patient I. Approximately 90% (by integration) of this iPTH consisted of COOH-terminal fragments of the hormone not recognized by the anti-N assay, a finding consistent with many other reports (6, 8, 10, 11, 13).

DISCUSSION

Although several reports describe multiple fragments of iPTH (principally COOH-terminal fragments) in

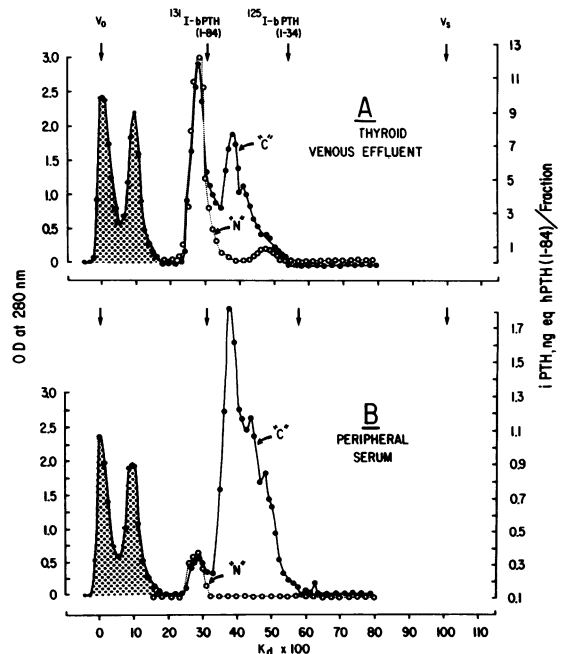


FIGURE 3 Gel filtration of 1.8-ml samples of serum from patient I (see Table I for details). The iPTH in the column fractions was determined with the anti-N and anti-C assays. Methods as in Fig. 1B. A, thyroid venous effluent serum. B (note difference in scale from A), peripheral serum.

the plasma of hyperparathyroid man, data in this paper show that PTH fragments are released by parathyroid tumors *in vivo*. Less than $\approx 10\%$ of the iPTH we observed, in thyroid or parathyroid venous effluents, could have been derived from the iPTH in peripheral plasma. These results confirmed the predictions of Silverman and Yalow (8), and Hesch et al. (39) but are not consistent with those of Potts et al. (9, 16–19) who concluded that nearly all of the iPTH secreted by parathyroid tumors consists of intact hormone.³ We speculate that our ability to demonstrate iPTH fragments in thyroid and parathyroid venous effluent serum is due to the inherently higher resolving power of our fractionation system than that used by Habener et al. (15).

A question immediately raised by our findings concerns the relative importance of contributions by two processes to the immunoheterogeneity of PTH in plasma: (a) peripheral degradation of secreted hPTH(1-84) to COOH-terminal fragments; and (b) direct glandular release of these fragments. Unfortunately, this question cannot be answered easily because precise information is not available about the fractional rates of disappearance of the various circulating forms of iPTH. Silverman and Yalow (8) have estimated that only 1/15th of the total secretory output of the parathyroid glands of a patient with secondary hyperparathyroidism due to renal failure would be required to account for 100% of the COOH-terminal fragments in this patient's peripheral blood. This estimate is based on a measured $t_{1/2}$ for these fragments 150 times greater than that of intact PTH. Available published data suggests that, in patients with primary hyperparathyroidism and normal renal function, the $t_{1/2}$ of endogenously-generated circulating COOH-terminal fragments is at least 5–10 times that of intact PTH (5, 8, 14) and it is generally agreed that, under steady-state conditions, the quantity of COOH-terminal PTH fragments in the serum of these patients exceeds that of intact PTH by 5- to 10-fold.

Whereas Potts et al. (9, 20, 21) have reported that endogenously administered labeled (with ¹²⁵I) or unlabeled PTH(1-84) is rapidly and specifically cleaved in normal cows, dogs, and rats, it is difficult to determine precisely from their data the percentage of the dose which was metabolized and appeared in the peripheral circulation as COOH-terminal fragments of PTH. Therefore, we are unable to say anything about the significance of peripheral metabolism of secreted PTH(1-84) to the pool of COOH-terminal PTH fragments in hyperparathyroid man.

On the other hand, our data showing that 20–50% of the total iPTH secreted by hyperfunctioning para-

thyroid glands consists of COOH-terminal fragments strongly suggest that the contribution of gland secretion of fragments to the immunoheterogeneity of PTH in the peripheral circulation is large. If the secretion rates of intact and fragment PTH are assumed to be constant and the fragments have a $t_{1/2}$ five times greater than that of the intact hormone, the contribution of secreted fragments to the iPTH in the peripheral circulation would be up to 2.5 times the quantity of intact hormone; if the fragments have a $t_{1/2}$ 10 times that of intact PTH, the contribution might be as large as five times the quantity of intact hormone. Therefore, it would appear that gland secretion could contribute 20–100% of the COOH-terminal fragments in the plasma of patients with primary hyperparathyroidism, if we assume a maximum excess of COOH-terminal fragments over intact PTH of only fivefold. In actuality, our data (Table II) suggest that the ratio of COOH-terminal fragments to intact PTH in peripheral serum ranges from 2 to at least 10.

It is probably safe to say that neither peripheral metabolism of intact PTH nor glandular secretion of COOH-terminal fragments is the exclusive source of the immunoheterogeneity of plasma PTH. However, as implied from our discussion of our data, we favor glandular secretion of fragments as the most important of the two sources in hyperparathyroidism. This conclusion must be conditioned by the assumption that the COOH-terminal fragments derived from glandular secretion or from peripheral metabolism of secreted PTH(1-84) have similar rates of disappearance. Our immunochemical data do not permit us to be certain that the COOH-terminal fragments which we have identified in thyroid or parathyroid venous effluents are the same as those present in the peripheral serum.

Earlier reports from our laboratory (11, 38) and several others (8, 12, 13) have established that there are at least two general types of PTH radioimmunoassays which have different utilities in the diagnosis of parathyroid dysfunction. We found that anti-N assays are most useful in studies of acute secretory dynamics of parathyroid tissue in response to hyper- or hypocalcemic stimuli (41). As well, this type of assay appears to be better than anti-C assays in the examination of blood samples obtained during catheterization of small veins of the neck and mediastinum for the purpose of localizing hyperfunctioning parathyroid tissue (38, 42). The results presented in this paper provide an explanation for this observation: we have measured a two- to fourfold greater step-up increase of iPTH in parathyroid or thyroid venous effluents with an anti-N assay than with an anti-C assay because of the lower concentration of iPTH measured with the anti-N assay in peripheral serum. Anti-C assays, on the other hand, have been shown to be superior when applied to the measurement of steady-state levels of iPTH for the

³ Mayer et al. (40) have reported (in abstract form) that COOH-terminal PTH fragments are secreted by parathyroid glands of hypercalcemic cows.

purpose of identifying states of chronic parathyroid hyperfunction (13, 38). The reasons for this superiority are not known. However, our studies provide a possible explanation: if parathyroid tumors secreted more COOH-terminal PTH fragments, or a higher proportion of these fragments, (or both), than normal tissue, these peptides might be considered "markers" for parathyroid hyperfunction which could be detected better by anti-C assays. Firm evidence confirming the paucity of or total absence of these PTH fragments in fractionated sera obtained directly from normal parathyroid glands would be required to support this hypothesis.

The existence of biologically active PTH fragments in bovine parathyroid tissue has been recognized for many years (43, 44). In demonstrating the presence of PTH fragments in extracts of human parathyroid tumors, Silverman and Yalow concluded that these fragments were artifacts originating from degradation of PTH(1-84) during tissue storage or extraction (8). However, we have recently found that large quantities of biologically inactive, crude COOH-terminal PTH fragments (suitable for producing PTH antisera directed toward the COOH-terminal region of hPTH) can be readily recovered from pooled, frozen human parathyroid tumors (45), and the results presented in this paper have now shown that COOH-terminal PTH fragments are secreted. One missing piece of information remains before these two observations can be firmly linked: the definitive demonstration that these fragments exist in human parathyroid tissue *in vivo*. Attention must also be directed toward the physiologic mechanisms for the elaboration of these PTH fragments and the possible roles played by calcium-regulated enzymes of the parathyroid gland (46). Other key questions relate to the control of secretion of these fragments and whether derangements in processes connected with PTH degradation in the parathyroid gland (47) might be a reflection of, or even contribute directly to, the pathogenesis or parathyroid gland dysfunction.

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