

Direct Release of Parathyroid Hormone Fragments from Functioning Bovine Parathyroid Glands In Vitro

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ABSTRACT To determine the origin of circulating parathyroid hormone fragments, hormonal peptides released from bovine parathyroid tissue in a physiologically responsive in vitro "perfusion" system were analyzed by gel exclusion chromatography and region-specific radioimmunoassays. When exposed to low Ca^{++} , the tissue released large quantities of intact hormone (parathyroid hormone 1-84) as well as amino- and carboxyl-terminal fragments. Fragments of the hormone were also released when the tissue was exposed to high Ca^{++} , but the carboxyl fragments comprised a much greater proportion of the hormonal peptides released. Control experiments indicated that fragmentation of the hormone occurred within the gland and not after it was secreted. These experiments provide direct evidence, therefore, that release of fragments from the parathyroid gland may contribute to the immunologic heterogeneity of the hormone in the circulation.

INTRODUCTION

Although the reciprocal relationship between secretion of parathyroid hormone (PTH)¹ and the serum Ca^{++} has been well documented (1), there has been uncertainty concerning the variety of hormonal fragments released from the glands in normal physiologic states (2-4). The intact hormone synthesized in the bovine parathyroid gland is an 84 amino acid single chain polypeptide released from cells in response to low calcium stimula-

tion (5). Habener et al. (4) and Segre et al. (6) provided early evidence that this was the predominant form of PTH found in parathyroid venous effluent blood. In peripheral blood, however, the major form of circulating PTH consists of carboxyl(COOH)-terminal fragments of the hormone, with only small amounts of intact 1-84 peptide present (6).

Experiments both in vivo (7, 8) and in vitro (9-11) have provided strong evidence that metabolic alteration of intact PTH after release from the gland is a significant factor in the production of circulating fragments of the hormone. Earlier in vitro studies (12, 13) had provided evidence for release of smaller molecular forms of PTH from parathyroid tissue, but the possibility of post-secretory cleavage of the hormone could not be completely ruled out in these short term tissue culture experiments. Flueck et al. (14) returned attention to the parathyroid gland as a source of the immunologic heterogeneity of PTH in plasma by demonstrating that the venous effluent of hyperfunctioning human parathyroid glands contained large quantities of COOH- and amino(NH_2)-terminal fragments of the hormone. Mayer and co-workers (15) also demonstrated an increased percentage of fragmentation of the hormone in parathyroid venous effluent when calves were made hypercalcemic. The availability in our laboratory of a functioning parathyroid gland "perfusion" system that responds to physiologic regulators (16) provided an opportunity to examine directly the nature of the hormone released from the tissue in this system.

METHODS

Perfusion system. The system used was similar to that described by Oldham et al. (17) but is based on the perfusion system of Stachura (18). Bovine parathyroid glands were obtained at a local abattoir within minutes of slaughter of the animal, placed in Ham's F10 nutrient medium (Difco Laboratories, Detroit, Mich.) and transported at 4°C to the laboratory. The glands were trimmed of fat and connective tissue, cut into 1-2-mm³ pieces, and preincubated for 2 h in 25-ml Erlenmeyer flasks that contained 8 ml Krebs-Ringer

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¹Abbreviations used in this paper: bPTH, bovine parathyroid hormone; COOH, carboxyl; K_d , partition coefficient; NH_2 , amino; PTH, parathyroid hormone.

bicarbonate buffer (pH 7.4), with 200 mg/dl glucose and 5% fetal calf serum. Preincubation was performed in a metabolic shaker at 37°C under an atmosphere of 5% CO₂-95% O₂. Ca⁺⁺ concentration was 1.5 mM and magnesium concentration was 0.75 mM during preincubation in all experiments. About 25 mg wet weight of tissue was then transferred to 0.2-ml microchambers that were perfused continuously with Krebs-Ringer bicarbonate buffer at a rate of 0.2 ml/min, samples being collected in 15-min aliquots (16). The medium was bubbled with 95% O₂-5% CO₂ for 1 h before adding the fetal calf serum and adjusting the pH to 7.4. Thereafter, an atmosphere of 5% CO₂-95% O₂ was maintained over the medium as it was pumped into the system and its pH remained between 7.35 and 7.45. Perfusate was collected in 13 × 100-mm test tubes on ice within 45 s after it left the tissue chamber. After completion of each experiment, the perfusate was frozen and stored at -20°C, and the tissue was homogenized in 8 M urea-0.2 N HCl and incubated at 4°C for 16 h. The homogenate was then centrifuged at 10,000 *g* to remove particulate matter and the protein content of 20 μ l of supernate determined by the method of Lowry et al. (19). The use of urea did not alter the measurement of protein significantly because it was corrected for in the standard curve. Hormone in the perfusate samples was measured by radioimmunoassay and the results expressed as quantity of hormone (nanograms) released per milligram of tissue protein.

Radioimmunoassay. The radioimmunoassays were carried out by the double-antibody method described previously (20). Two region-specific radioimmunoassays were used for analysis of perfusate medium and gel chromatography fractions. The first involved a highly specific COOH-terminal antiserum which was developed by repeated immunizations of guinea pigs with parathyroid gland trichloroacetic acid powder (Inolex Corp., Biomedical Div., Glenwood, Ill.) in Freund's adjuvant by the method of Vaitukaitis et al. (21). Before immunization, the hormonal powder was coupled with carbodiimide to polylysine, and the conjugate was injected into Strain 2 animals (22, 23). To determine the antigenic specificity of the antiserum, it was tested with intact bovine PTH (bPTH) (highly purified grade, Inolex Corp.), synthetic NH₂-terminal 1-34 bPTH (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) and purified 53-84 bovine COOH-terminal peptide (obtained through the generosity of Doctors Henry Keutmann and John T. Potts, Jr.). The second radioimmunoassay, highly specific for the NH₂-terminal region of PTH, was developed by repeated immunization with synthetic 1-34 bPTH and was generously supplied by Doctors David Ontjes and Samuel Wells. Radioiodinated 1-84 bPTH was used for both the COOH- and NH₂-terminal assays. bPTH 1-84 and bPTH 1-34 peptide were radioiodinated with the chloramine T method (24). Standards for the assays were prepared from the same 1-84 bPTH used for iodination.

Gel exclusion chromatography. Samples were chromatographed on 200-400 mesh Bio-Gel P-10 (Bio-Rad Laboratories, Richmond, Calif.), in a 95 × 1.2-cm column with 0.15 M ammonium acetate (pH 5.0) buffer that contained 5% fetal calf serum. The calf serum at this dilution contained undetectable concentrations of PTH. Sample size applied to the column was 0.8 ml, and flow rate was 4.5 ml/h. 1-ml fractions were collected, lyophilized, and reconstituted for radioimmunoassay in 0.8 ml 0.01 M sodium barbital buffer (pH 8.6) that contained 0.01 M EDTA and 16% by volume PTH-free human plasma. Insignificant quantities of ammonium acetate remained after lyophilization and did not affect the radioimmunoassays. Even numbered column fractions were assayed with one region-specific antiserum and odd-numbered fractions with the other. The column was calibrated with ¹³¹I-bPTH 1-84 and ¹²⁵I-synthetic bPTH 1-34. Partition coef-

ficients (*K_d*) for radioactive and immunoreactive peaks were calculated by the formula $K_d = V_e - V_0 / V_s - V_0$, where *V₀* was the void volume, *V_e* the elution position of the peak, and *V_s* the salt volume (determined by the elution position of [¹⁴C]leucine).

Preparation of [¹⁴C]PTH. Bovine parathyroid tissue was incubated for 2 h in Krebs-Ringer bicarbonate buffer that contained 10 μ Ci/ml [¹⁴C]leucine (New England Nuclear, Boston, Mass.). Trichloroacetic acid powder was prepared from the tissue by the method of Chu et al. (25), with further purification by the method of Keutmann et al. (26) with chromatography on Sephadex G-100 (Pharmacia Fine Chemicals Inc., Piscataway, N. J.). A single peak of [¹⁴C]PTH coeluted with immunoreactive PTH and was rechromatographed on the same Bio-Gel P-10 column used for analysis of the perfusate samples. The ¹⁴C-labeled hormone also appeared as a single peak in the position of PTH on polyacrylamide gel electrophoresis at pH 4.4. Labeled hormone was added to the perfusion medium in the presence or absence of parathyroid tissue to determine whether hormonal fragments were generated extracellularly.

RESULTS

Radioimmunoassays. Critical to evaluation of the type of hormone released from the gland was the use of region-specific radioimmunoassays. As demonstrated in Fig. 1, antiserum GP TCA-5 was highly specific for the COOH-terminal region of the hormone. At the titer used in the assay (1:20,000), the antiserum recognized only an antigenic determinant within the 53-84 sequence of bPTH. Equivalent molar quantities of the 1-84 and 53-84 peptides competed equally for displacement of ¹³¹I-PTH binding to antibody. The antiserum did not recognize the NH₂-terminal 1-34 fragment at all. The specificity of the NH₂-terminal antiserum is demonstrated in Fig. 2. This antiserum was generated against the 1-34 peptide and had no cross-reactivity against the 53-84 fragment. In contrast to the COOH-terminal antiserum, which showed equimolar sensitivity for the fragment and intact hormone, GP-Cl (at a titer of 1:20,000) was more sensitive to the fragment than intact hormone, by several fold. This difference in reactivity would tend to overestimate the concentration of NH₂-terminal fragment relative to intact PTH.

Gel filtration studies. The gel filtration column used for each experiment was standardized with radioiodinated 1-84 PTH and 1-34 fragment. Their positions are indicated in Fig. 3. The *K_d* for 1-84 bPTH (both radioiodinated and immunoreactive) was 0.22 and for 1-34 PTH, 0.65. Their elution positions were constant and were checked after new samples of perfusion medium were chromatographed. Iodinated PTH was not cochromatographed with the samples tested. Highly purified bPTH was passed through the column and assayed for both NH₂ and COOH-terminal activity. The two region-specific antisera each recognized a single peak of intact hormone; the peaks were superimposable and the *K_d* was the same as that of ¹³¹I-bPTH 1-84.

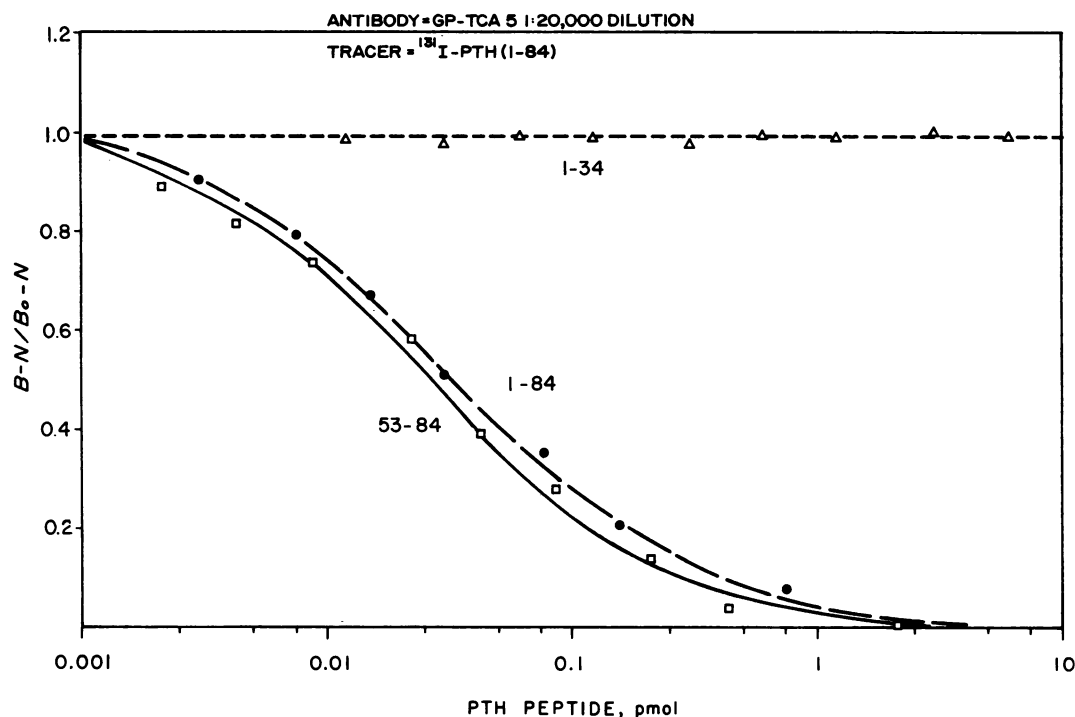


FIGURE 1 Standard curve for COOH-terminal radioimmunoassay comparing reactivity of intact bPTH 1-84 and bPTH 53-84 with antibody (GP-TCA5) generated against bPTH 1-84. Radioiodinated bPTH 1-84 was used as tracer, and experimental counts bound (B) minus non-specific precipitation (N) divided by control counts (B_0-N) is plotted against the log of PTH or fragment given in moles. Note complete failure of synthetic bPTH 1-34 to react with the antibody, and the equimolar responses of bPTH 1-84 and 53-84 fragment.

To be certain that hormonal fragments in the perfusion effluent medium were not generated after leaving the tissue, purified [¹⁴C]PTH was passed through the perfusion system in the presence of tissue. When labeled hormone was passed through the perfusion system in medium that contained 2.5 mM Ca⁺⁺, a homogeneous peak at the position of intact PTH was noted on gel filtration (see Fig. 3). This was the same pattern noted for ¹⁴C-labeled hormone that was not passed through the system. Identical results were obtained under low Ca⁺⁺ (0.5 mM) conditions and with radioiodinated hormone (not shown). These findings demonstrated that the fragments observed in the medium were not produced after release of hormone into the chamber but were derived from the tissue itself. No difference was noted between the elution pattern of [¹⁴C]PTH which had been perfused through the system and left standing in perfusion medium at room temperature for 1 h, and [¹⁴C]PTH in medium that was collected on ice during the experiment. The pattern shown in Fig. 3 is actually that of the material collected at room temperature.

Physiologic studies. To document the physiologic responsiveness of the tissue in the perfusion system, experiments were performed in which the tissue was

initially exposed to high Ca⁺⁺ medium for 1 h followed by exposure to low Ca⁺⁺ for an additional 90 min (Fig. 4). The change from high (2.0 mM) to low (0.5 mM) Ca⁺⁺ led to a marked increase in hormone release (as assessed both by NH₂- and COOH-terminal assays). The concentration of hormone assessed by both assays at low Ca⁺⁺ was identical, but at high Ca⁺⁺ more COOH- than NH₂-terminal activity was noted. To determine more precisely the nature of the hormone released during high and low Ca⁺⁺ perfusion, samples were subjected to gel filtration and the fractions assessed by region-specific assay. The hormone released at low Ca⁺⁺ concentration, when assayed for COOH-terminal activity, showed a dominant peak in the position of intact hormone followed by at least two smaller peptides that contained COOH-terminal determinants with K_d values of 0.35 and 0.49, respectively (Fig. 5A). Analysis of the same sample with the NH₂-terminal assay (Fig. 5B) indicated a peak of intact hormone identical to that determined by the COOH-terminal assay and a large peak of NH₂-terminal activity which eluted with a K_d of 0.49, at a position later than the major peak of COOH-terminal activity, but before ¹²⁵I-bPTH 1-34.

When the hormone released during high Ca⁺⁺ perfusion was assessed by the same methods there was a

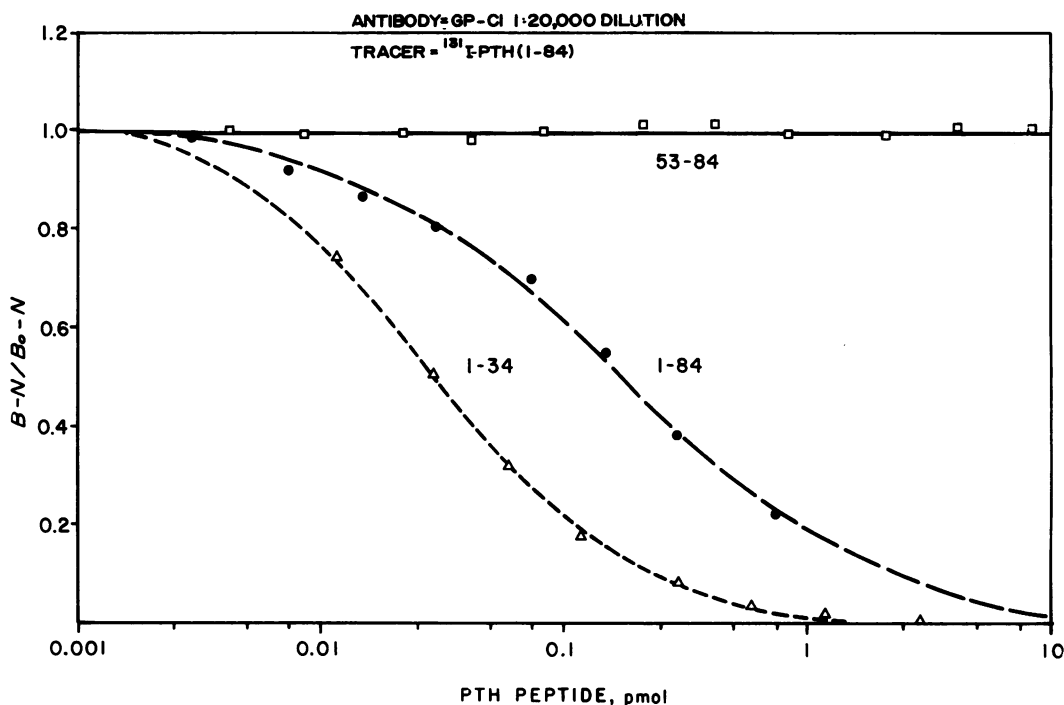


FIGURE 2 Standard curve for NH_2 -terminal radioimmunoassay comparing immunoreactivity of intact bPTH 1-84 and bPTH 1-34 with an antibody (GP-C1) generated against PTH 1-34. Radioiodinated bPTH 1-84 was used as tracer. Note failure of bPTH 53-84 to displace labeled hormone and enhanced immunoreactivity of bPTH 1-34 compared with bPTH 1-84 in the assay.

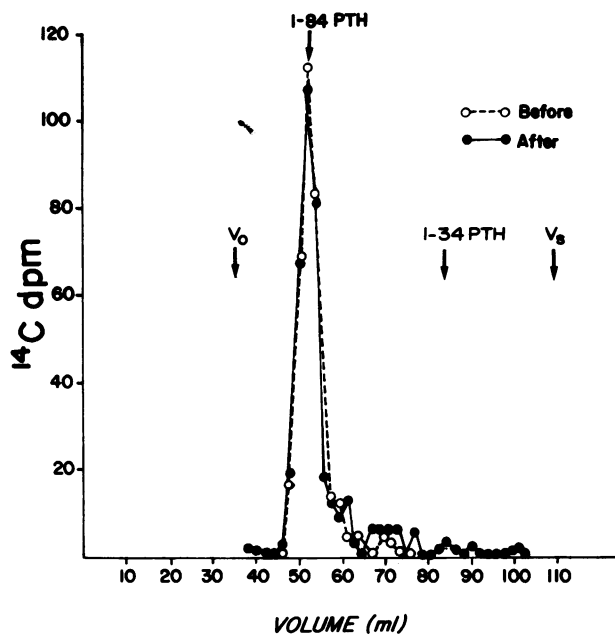


FIGURE 3 Control experiment in which purified ^{14}C -labeled PTH was chromatographed on Bio-Gel P-10 before and after perfusion through system with tissue at 2.5 mM Ca^{++} . The elution positions of ^{125}I -labeled bPTH 1-84, ^{125}I -labeled bPTH 1-34, void volume (V_0), and the salt peak (V_s) (^{14}C]leucine) are noted.

striking decrease in the proportion of intact hormone (Fig. 6). Of interest was the marked increase and dominance of the peak having only COOH-terminal immunoreactivity ($K_d = 0.35$) after the intact hormone, and the presence of a second peak at $K_d = 0.49$ of a peptide or peptides with both COOH and NH_2 -terminal determinants.

DISCUSSION

Heterogeneity of polypeptide hormones in plasma has been a subject of considerable interest in recent years. The most extensive work in this area has involved PTH and its fragments, with studies focusing on the nature of circulating fragments and their potential sites of origin (2, 3, 6, 27). The early observations of Berson and Yalow (27) provided clear evidence of heterogeneity of PTH in peripheral blood. Subsequent *in vitro* studies in two laboratories suggested that PTH in short-term tissue culture medium had a lower molecular weight and an immunologic activity different from the hormone extracted from the gland (12, 13). Although Martin et al. (28) suggested that the latter findings resulted from degradation of hormone after release into the medium, the question of direct glandular release of fragments remained unsettled. Habener et al. (4) then examined PTH in venous effluent from the glands of normal cows

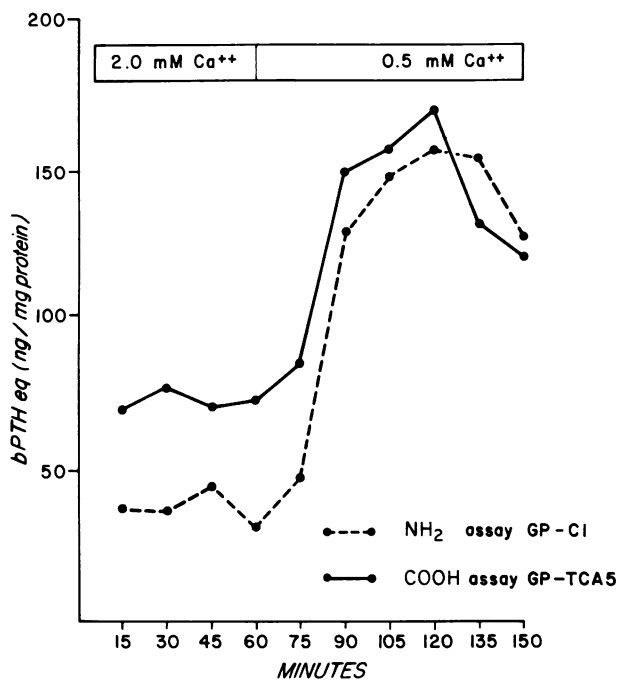


FIGURE 4 Release of PTH from perfusion system during high Ca^{++} suppression (2.0 mM) followed by low Ca^{++} stimulation (0.5 mM). Similar physiologic responses were obtained with NH_2 - and COOH-terminal radioimmunoassays, although COOH-terminal activity was higher than NH_2 -terminal activity during high Ca^{++} . Results expressed as nanograms PTH released per milligram tissue protein.

and compared it with hormone in the peripheral blood. In these studies, and in subsequent ones by Segre et al. (6), in man it was observed that hormone in gland effluent was principally intact, whereas smaller COOH-terminal fragments of the hormone predominated in peripheral blood. These authors suggested that fragmentation of the molecule occurring after secretion was the principal source of the circulating forms of PTH. In subsequent studies (7, 8), it was confirmed that fragmentation of hormone occurred after secretion, because intact hormone injected into the peripheral blood was rapidly cleaved into fragments. This still left no definitive resolution to the question of glandular release of fragments.

With the use of kinetic arguments, Silverman and Yalow (3) suggested that fragments of PTH might be released by the gland and persist in the peripheral blood because of a long half-life. Flueck et al. (14), with high-resolution gel chromatography of neck vein samples from patients with primary hyperparathyroidism, found large quantities of COOH- and NH_2 -terminal fragments of PTH that could not be explained by recirculation of peripherally metabolized PTH 1-84. Strongly supportive data were also presented by Mayer et al. (15), who demonstrated that neck-vein effluent of calves contained COOH-terminal fragments. In fact, during experimental hypercalcemia more fragments than intact hormone were released from the gland. Fragments of PTH have also been extracted

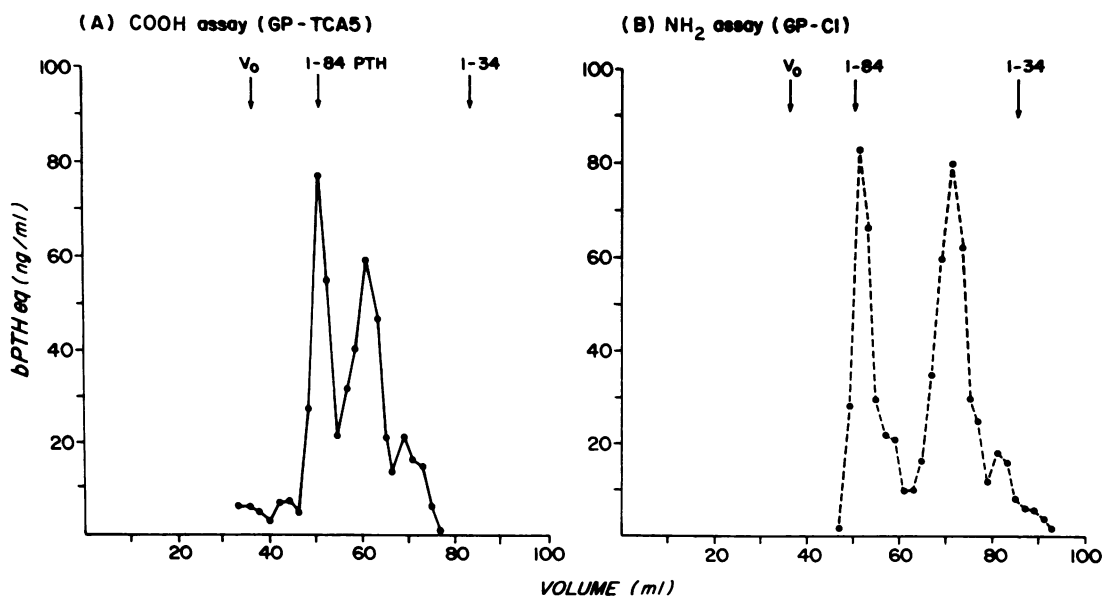


FIGURE 5 Representative analysis of samples from low (0.5 mM) Ca^{++} perfusion over gel filtration P-10 column by COOH- (A) and NH_2 - (B) terminal specific assays. Evidence for both COOH- and NH_2 -terminal fragments was present. Quantity of intact hormone (at elution position of 1-84 PTH) was similar by both NH_2 - and COOH-terminal assays. Assay results expressed as equivalents of bPTH (1-84) in nanograms per milliliter.

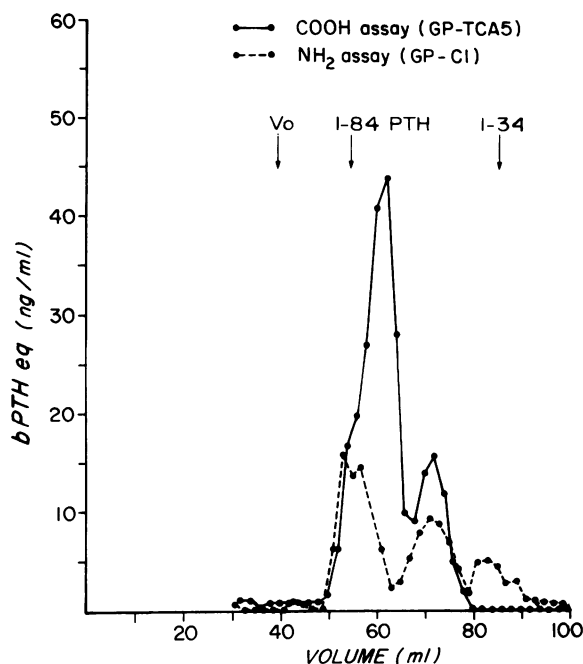


FIGURE 6 Representative analysis of samples from high (2.0 mM) Ca^{++} perfusion over Bio-Gel P-10 column by COOH and NH_2 -terminal assays. Decrease in release of intact hormone (compared with Fig. 5) by NH_2 and COOH assays with a greater proportion of COOH-terminal fragments was present. Assay results expressed as equivalents of (bPTH 1-84) in nanograms per milliliter.

from both human and bovine parathyroid tissue (3, 29, 30), but it remains to be demonstrated that these fragments are the same ones seen in the circulation.

Because of the controversies surrounding the question of fragment release as well as the availability of a physiologically functioning *in vitro* system, we have re-examined critically the earlier suggestion that smaller hormonal forms might be released from the gland (12, 13). The *in vitro* perfusion "model" has the distinct advantage of avoiding continued contact between secreted hormone and the tissue which may have contributed to some of the fragmentation of PTH noted in earlier studies. From the current work, the following evidence in favor of direct release of hormonal fragments can be cited: (a) Fragments of PTH were present in perfusion medium that was sampled within seconds after contact with the gland. (b) Substantial evidence against postsecretory fragmentation of the hormone was provided by control experiments in which ^{14}C - and ^{131}I -labeled hormones were run through the perfusion system, and fragmentation was not observed. (c) The degree of fragmentation varied in proportion to the concentration of Ca^{++} , with proportionally less intact hormone and more fragments being observed at high Ca^{++} . (d) The use of region-specific radioimmunoassay and standardized columns confirmed the presence of fragments in all

samples studied. The degree of fragmentation was unrelated to the time of the experiment from which the sample was obtained (after 30 min or up to 4 h of perfusion). (e) The positions of elution of the major fragments, both COOH- and NH_2 -terminal, were constant in more than 20 separate experiments.

Generation of the fragments in our studies seems to occur within the gland itself. Earlier studies by Fischer et al. (31) demonstrated calcium-dependent degradation of PTH by parathyroid tissue homogenates. Peptidase activity in the parathyroid glands was responsible for converting 1-84 PTH to smaller discrete fragments, with the major fragment having biologic activity. This enzyme was found in high concentration in the liver as well and in lower concentrations in the spleen, pancreas, and adrenal glands, but it was activated by low rather than high Ca^{++} . Under high Ca^{++} conditions, marked cleavage of the hormone to small fragments was noted. Canterbury et al. (10) found increased production of hormone fragments by isolated-perfused rat liver when a lower Ca^{++} concentration was used in the perfusing fluid. Hruska et al. (32) also found increased degradation of PTH by the isolated perfused kidney at low Ca^{++} concentration. In contrast, our studies showed proportionally increased fragment release at higher concentrations of Ca^{++} . Habener et al. (33) also suggested that high Ca^{++} , in addition to suppressing hormone release, regulated the amount of hormone available for secretion by causing accelerated intracellular degradation of PTH. The current studies are consistent with that suggestion. When they are coupled with recent *in vivo* observations in man (14) and the cow (15), they provide strong evidence for direct release of fragments of PTH from the parathyroid gland.

Although we examined the nature of hormone released from the gland in earlier studies (20, 34) and suggested that intact hormone was the predominant form released, fragment release in these studies was definitely not ruled out and was even suggested in one (34). The samples studied were obtained under low calcium stimulation in which a smaller proportion of fragments was released, and separate NH_2 - and COOH-terminal assays were not performed. The current report represents a much more systematic and detailed approach to the study of fragments. In the earlier work, evaluation of physiologic responses of normal and abnormal tissue was the primary goal.

Quantitation of hormone fragments is a difficult area, particularly because their amino acid sequence is not known. If 1-34 bPTH were a true fragment, its presence would tend to be overestimated in the NH_2 -terminal assay system with 1-84 PTH as tracer and in standard (see Fig. 2). It is less likely that the COOH-terminal antiserum would overestimate fragments because of the equimolar activity of 1-84 and 53-84 peptide. In the present study, we prefer to interpret our results only qualitatively, indicating that the parathy-

roid tissue is capable of releasing both COOH- and NH₂-terminal peptides. On the basis of over 20 separate experiments, the higher percentage of fragmentation at high calcium concentration is a consistent finding. Further studies of the precise chemical nature and quantity of specific hormonal fragments is needed.

The physiologic basis for release of hormonal fragments by the gland is unknown. On the one hand, it could represent an important physiologic control mechanism; on the other hand, it could represent non-specific release of fragments after intracellular hormone degradation. If intracellular cleavage is Ca⁺⁺-dependent and principally operative on intracellular stores, release of fragments could be explained as follows: when the cell is exposed to low calcium, PTH in storage granules is released. New hormone synthesis is stimulated, and the newly-synthesized PTH is rapidly transported through the cytoplasm, bypassing tissue storage, and possibly bypassing to a greater degree the degradative pathway (35). This would result in an increased proportion of intact hormone being secreted. At high Ca⁺⁺ concentration, the stimulus to secretion is decreased, although not completely (36, 37), and proportionally more fragmentation and hormone degradation would occur. Either specific or nonspecific release of fragments would then contribute to heterogeneity of the hormone in the circulation.

The varying origin of fragments in peripheral blood as well as the different rates of turnover make analysis and interpretation of hormone in a single sample of peripheral blood extraordinarily complex. From the above discussion, it appears that fragments could be both released from the gland and generated postsecretion in kidney, liver, bone, and possibly other sites (9–11). Whether the fragments generated from the gland are the same fragments generated in the periphery is unknown. Segre et al. (7) provided strong experimental evidence for cleavage of the bovine hormone after secretion between amino acids 33 and 34 and between 36 and 37. The fragments of PTH generated in our system have not been characterized further biochemically, but the major COOH-terminal fragment elutes at the same position as COOH-terminal fragments in peripheral plasma. As shown in Fig. 6, the major NH₂-terminal fragment eluted earlier than 1–34 PTH and conceivably could be similar to the 1–65 fragment extracted from bovine parathyroid glands by Murray et al. (30). Further studies of these fragments with region-specific antisera to different portions of the bovine molecule and purification of the fragments for chemical analysis are necessary to pursue these questions further.

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REFERENCES

1. Sherwood, L. M., G. P. Mayer, G. F. Ramberg, Jr., D. S. Kronfeld, G. D. Aurbach, and J. T. Potts, Jr. 1968. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. *Endocrinology*. **83**: 1043–1051.
2. Reiss, E., and J. M. Canterbury. 1974. Emerging concepts of the nature of circulating parathyroid hormones: implications for clinical research. *Recent Prog. Horm. Res.* **30**: 391–429.
3. Silverman, R., and R. S. Yalow. 1973. Heterogeneity of parathyroid hormone. Clinical and physiologic implications. *J. Clin. Invest.* **52**: 1958–1971.
4. Habener, J. F., D. Powell, T. M. Murray, G. P. Mayer, and J. T. Potts, Jr. 1971. Parathyroid hormone: secretion and metabolism *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* **68**: 2986–2991.
5. Habener, J. F. 1976. New concepts in the formation, regulation of release and metabolism of parathyroid hormone. In *Polypeptide Hormones: Molecular and Cellular Aspects*. CIBA Foundation Symposium 41. Excerpta Medica, Elsevier North Holland, Amsterdam. 197–224.
6. Segre, G. F., J. F. Habener, D. Powell, G. W. Tregear, and J. T. Potts, Jr. 1972. Parathyroid hormone in human plasma. Immunochemical characterization and biological implications. *J. Clin. Invest.* **51**: 3163–3172.
7. Segre, G. V., H. D. Niall, J. F. Habener, and J. T. Potts, Jr. 1974. Metabolism of parathyroid hormone. Physiologic and clinical significance. *Am. J. Med.* **56**: 774–784.
8. Hruska, K. A., R. Kopelman, W. E. Rutherford, S. Klahr, and E. Slatopolsky. 1975. 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.
9. Fujita, T., H. Orimo, M. Ohata, M. Yoshikawa, and M. Maruyama. 1970. Enzymatic inactivation of parathyroid hormone by rat kidney homogenate. *Endocrinology*. **86**: 42–49.
10. Canterbury, J. M., L. A. Bricker, G. S. Levey, P. L. Kozlovskis, E. Ruiz, J. E. Zull, and E. Reiss. 1975. Metabolism of bovine parathyroid hormone. Immunological and biological characteristics of fragments generated by liver perfusion. *J. Clin. Invest.* **55**: 1245–1253.
11. Freitag, J., K. Martin, M. Conrades, and E. Slatopolsky. 1977. Metabolism of bovine parathyroid hormone 1–84 by fetal rat calvaria. *Clin. Res.* **25**: 561A. (Abstr.)
12. Arnaud, C. D., H. S. Tsao, and S. B. Oldham. 1970. Native human parathyroid hormone: an immunochemical investigation. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 415–422.
13. Sherwood, L. M., J. S. Rodman, and W. B. Lundberg, Jr. 1970. Evidence for a precursor to circulating parathyroid hormone. *Proc. Natl. Acad. Sci. U. S. A.* **67**: 1631–1638.
14. Flueck, J. A., F. P. Dibella, A. J. Edis, J. M. Kehrwald, and C. D. Arnaud. 1977. Immunoheterogeneity of parathyroid hormone in venous effluent serum from hyperfunctioning parathyroid glands. *J. Clin. Invest.* **60**: 1367–1375.
15. Mayer, G. P., J. A. Keaton, J. G. Hurst, and J. F. Habener. 1977. Effects of plasma calcium concentration on the relative proportion of hormone and carboxyl-fragment in parathyroid venous blood. In *Abstracts of the 59th Annual Meeting of the Endocrine Society, Chicago*. 355. (Abstr.)

16. Sherwood, L. M., D. A. Hanley, K. Takatsuki, M. E. Birnbaumer, A. B. Schneider, and S. A. Wells, Jr. 1978. Regulation of parathyroid hormone secretion. In *Endocrinology of Calcium Metabolism. Proceedings of the Sixth Parathyroid Conference*, Vancouver, Canada. 12-17 June 1977. Excerpta Medica, Amsterdam. 301-307.
17. Oldham, S. B., J. A. Fischer, C. C. Capen, G. W. Sizemore, and C. D. Arnaud. 1971. Dynamics of parathyroid hormone secretion *in vitro*. *Am. J. Med.* **50**: 650-657.
18. Stachura, M. E. 1976. Basal and dibutyl cyclic AMP stimulated release of newly synthesized and stored growth hormone from perfused rat pituitaries. *Endocrinology*. **98**: 580-589.
19. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
20. Schneider, A. B., S. A. Wells, Jr., J. C. Gunnells, J. B. Leslie, and L. M. Sherwood. 1977. Regulation of function of transplanted parathyroid glands in man. *Am. J. Med.* **63**: 710-717.
21. Vaitukaitis, J., J. B. Robbins, E. Nieschlag, and G. T. Ross. 1971. A method for producing specific antisera with small doses of immunogen. *J. Clin. Endocrinol. Metab.* **33**: 988-991.
22. Goodfriend, T. L., L. Levine, and G. D. Fasman. 1964. Antibodies to bradykinin and angiotensin. A use of carbodiimides in immunology. *Science (Wash. D. C.)*. **144**: 1344-1346.
23. Schlossman, S. F., A. Yaron, S. Ben-Efraim, and S. A. Sober. 1965. Immunogenicity of a series of α , N-DNP-L-lysines. *Biochemistry*. **4**: 1638-1645.
24. Greenwood, F. C., W. M. Hunter, and J. S. Glover. 1963. The preparation of ^{131}I labelled human growth hormone of high specific radioactivity. *Biochem. J.* **89**: 114-123.
25. Chu, L. L. H., R. R. MacGregor, P. I. Liu, J. W. Hamilton, and D. V. Cohn. 1973. Biosynthesis of parathyroid hormone and parathyroid hormone by human parathyroid glands. *J. Clin. Invest.* **52**: 3089-3094.
26. Keutmann, H. T., G. D. Aurbach, B. F. Dawson, H. D. Niall, L. J. Deftos, and J. T. Potts, Jr. 1971. Isolation and characterization of the bovine parathyroid isohormones. *Biochemistry*. **10**: 2779-2787.
27. Berson, S. A., and R. S. Yalow. 1968. Immunochemical heterogeneity of parathyroid hormone in plasma. *J. Clin. Endocrinol. Metab.* **28**: 1037-1047.
28. Martin, T. J., P. B. Greenberg, and R. A. Melick. 1972. Nature of human parathyroid hormone secreted by monolayer cell cultures. *J. Clin. Endocrinol. Metab.* **34**: 437-440.
29. Dibella, F. P., J. B. Gilkinson, J. Flueck, and C. D. Arnaud. 1978. Carboxyl-terminal fragments of human parathyroid hormone in parathyroid tumors: unique new source of immunogens for the production of antisera potentially useful in the radioimmunoassay of parathyroid hormone in human serum. *J. Clin. Endocrinol. Metab.* **46**: 604-612.
30. Murray, T. M., S. A. Muzzafar, J. A. Parsons, and H. T. Keutmann. 1975. A biologically active hormonal fragment isolated from bovine parathyroid glands (BPTH 1-65). *Biochemistry*. **12**: 2705-2711.
31. Fischer, J. A., S. B. Oldham, G. W. Sizemore, and C. D. Arnaud. 1972. Calcium-regulated parathyroid hormone peptidase. *Proc. Natl. Acad. Sci. U. S. A.* **69**: 2341-2345.
32. Hruska, K. A., K. Martin, P. Mennes, A. Greenwalt, C. Anderson, S. Klahr, and E. Slatopolsky. 1977. Degradation of parathyroid hormone and fragment production by the isolated perfused dog kidney. *J. Clin. Invest.* **60**: 501-510.
33. Habener, J. F., B. Kemper, and J. T. Potts, Jr. 1975. Calcium-dependent intracellular degradation of parathyroid hormone: a possible mechanism for regulation of hormone stores. *Endocrinology*. **97**: 431-441.
34. Birnbaumer, M. E., A. B. Schneider, D. Palmer, D. A. Hanley, and L. M. Sherwood. 1977. Secretion of parathyroid hormone by abnormal human parathyroid glands *in vitro*. *J. Clin. Endocrinol. Metab.* **45**: 105-113.
35. MacGregor, R. R., J. W. Hamilton, and D. V. Cohn. 1975. The by-pass of tissue hormone stores during the secretion of newly synthesized parathyroid hormone. *Endocrinology*. **97**: 178-188.
36. Targovnik, J. H., J. S. Rodman, and L. M. Sherwood. 1971. Regulation of parathyroid hormone secretion *in vitro*: quantitative aspects of calcium and magnesium ion control. *Endocrinology*. **88**: 1477-1485.
37. Mayer, G. P., J. F. Habener, and J. T. Potts, Jr. 1976. Parathyroid hormone secretion *in vivo*. Demonstration of a calcium-independent non-suppressible component of secretion. *J. Clin. Invest.* **57**: 678-683.