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NOTE

1-34 Human Parathyroid Hormone Radioimmunoassay: Properties of Antiserum Against Synthetic 1-34 Human Parathyroid Hormone and Its Clinical Application

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Abstract

Antibody against synthetic 1-34 humanPTH synthesized by Niall et al. (1974), was developed in rabbits. Synthetic 1-34 hPTH was found to be a good immunogen for rabbit. The relative importance of various structural parts of 1-34 hPTH molecule with regard to immunological specificity was determined by reference to inhibition of specific binding of 125I-1-34 hPTH to the antibody by analogues of 1-34 hPTH (NLeu⁸-NLeu¹⁸ 1-34 hPTH, NLeu⁸-NLeu¹⁸-Tyr³⁴ hPTH) and by 1-34 bPTH. The immunological recognition site in 1-34 hPTH molecule was found to be located around the 8 to 18 amino acid sequence, because the binding affinity to the antibody of this analogue (NLeu⁸-NLeu¹⁸ 1-34 hPTH) was less than that of the native hormone in the antibody. Furthermore, other immunological recognition sites were located in the C- and N-terminal regions of this molecule, as reported by Segre et al. (1976) and Visser et al. (1979).

This antiserum could measure only 1-34 hPTH molecule in serum, since it did not crossreact with partially purified 1-84 hPTH.

In order to evaluate the advantage of 1-34 hPTH radio-immunoassay (RIA) in the diagnosis of parathyroid dysfunction, serum PTH levels in various diseases were measured by both 1-34 hPTH RIA and 1-84 PTH RIA and the values obtained by these assays were compared.

There was a good correlation between the values obtained by 1-34 hPTH RIA and those by 1-84 PTH RIA. However, in patients with chronic renal failure, the incidence of cases with high serum PTH level was 90% when measured by 1-84 PTH RIA while it was 39% when measured by 1-34 hPTH RIA. Serum PTH levels in primary hyperparathyroidism were abnormally high and those in hypoparathyroidism were low in both assays. Some patients with senile osteoporosis had a high serum PTH level. The incidences of cases with a high serum PTH level in this disease were equal in both assays.

In conclusion, these two site specific RIAs (1-34 PTH RIA and 1-84 PTH RIA) were useful in the evaluation of PTH secretion and/or metabolism.

It has been well known that parathyroid hormone (PTH) immunoreactivity in human plasma is shared by several more or less distinct fragments (Berson and Yallow 1968, Aurbach et al., 1972; Arnaud, 1973; Segre et al., 1974; Silverman and Yallow, 1973; Arnaud et al., 1974; Reiss and Canterbury, 1973; Flueck et al., 1977). Recently, a biologically active fragment comprising the first 34 amino acid residues of human parathyroid hormone (1-34 hPTH) has been synthesized (Niall et al., 1974; Keutman et al., 1975) and the specific and homologous radioimmunoassay systems for 1-34 hPTH were available in several laboratories (Desplan et al., 1977, 1978; Habener September 8, 1980.
et al., 1972; Flueck et al., 1977; Segre and Potts, 1976; Visser et al., 1979). However, little information has been made available on the immunological recognition sites in the amino acids sequence of 1-34hPTH molecule except for the report of Visser et al. (1975). Furthermore, scarcely any information was reported on the usefulness of serum 1-34hPTH radioimmunoassay in the diagnosis of disorders of parathyroid function.

We have recently raised a specific antiserum to 1-34hPTH synthesized by Niall et al. (1974), and checked the immunological properties of this antiserum and subsequently, developed a radioimmunoassay for serum PTH in order to evaluate its clinical usefulness in the diagnosis of parathyroid disorders.

Materials and Methods

1. 3-34 hPTH
1-34 human PTH, having the structure proposed by Niall et al. (1974) and Keutman et al. (1975) was kindly supplied by Dr. Bastian, Armours pharmaceutical Co., Illinois, USA. (lot No. K 744206-13).

2. PTH analogues
1-34 hPTH analogues, NLeu8-NLeu18-1-34 hPTH and NLeu8-NLeu18-Tyr34-1-34 hPTH were kindly supplied by Toyojozo pharmaceutical Co., Ohito, Japan. 1-34 bovine PTH (1-34 bPTH) was purchased from Beckman Co., Geneva, Switzerland (lot No. B0454). Highly purified 1-84 bPTH was purchased from Inolex Co., Illinois, USA and partially purified human PTH (lot. No. 75/549) was kindly supplied by the National Institute for Biological Standards and Control, Holly Hill, London, U. K.

3. Production of antiserum
White rabbit was immunized with 70 µg of 1-34 hPTH in a mixture of 0.1 M acetic acid and complete Freund's adjuvant (1:1, v/v). Eleven injections were given subcutaneously at multiple sites on the back at intervals of 2 weeks. The antiserum used in the present study was obtained 1 week after the last injection.

4. Radiiodination of 1-34 hPTH
1-34 hPTH was labelled with 125I according to the method of Hunter and Greenwood (1962). 125I-labelled 1-34 hPTH had a specific activity of about 130 µCi/µg.

5. Radioimmunoassay (RIA) procedure
100 µl of standard 1-34 hPTH or test substance and 100 µl of diluted antiserum were mixed and the final volume was adjusted to 400 µl with standard diluent (0.05 M veronal buffer pH 8.6, containing 0.5% BSA and 0.05% EDTA 2Na). Subsequently, the mixtures were incubated for 72 hours at 4°C and 125I-labelled 1-34 hPTH (approx. 8000 cpm, 100 µl) was added and the mixtures were incubated for 48 hours at 4°C. Separation of bound from free 125I-labelled 1-34 hPTH was carried out using the second antibody method or dextran coated charcoal method.

6. 1-84 PTH radioimmunoassay
1-84 PTH RIA kit was kindly supplied by Eiken Chemical Co., Ltd., Tokyo, Japan. This antiserum was raised against purified 1-84 bovine PTH (1-84 bPTH) in a guinea pig. The minimal detectable limit of this kit was 0.1 ng/ml and intra and interassay variances were 9.0% and 18.2%, respectively.

7. Patients
Serum PTH concentrations were measured in control subjects (n=12), patients with primary hyperparathyroidism (n=4; PHP), hypoparathyroidism (n=6, HP), chronic renal failure on hemodialysis (n=48; CRF), and senile osteoporosis (n=60).

Results

(1) The binding capacity of antiserum to 1-34 hPTH;
Rabbit injected with 1-34hPTH responded to the immunization by producing antibody. Antiserum named R-I showed significant binding with 125I-1-34hPTH at the final concentration of 1: 320,000.

(2) Standard curve of 1-34 hPTH RIA;
The minimal detectable limit of the RIA using antiserum R-I was 0.11 ng/ml at the final dilution of 1: 320,000. The standard curve of the 1-34hPTH RIA obtained by separate 6 experiments is shown in Fig. 1a. In this radioimmunoassay system, intra and interassay variance were less than 15%.
Figure 1. The standard curve of 1-34 hPTH radioimmunoassay using antisera R-I.
Initial B/T was 21.1±3.0% (n=6, R-I) at the final concentration of antisera 1:320,000 (R-I).
The minimal detectable limit was 0.11 ng/ml in this radioimmunoassay system. The vertical bars in figures represent mean±S.E.

(3) Crossreactivity of antiserum with 1-34hPTH with 1-34hPTH analogues and 1-34bPTH;
Crossreactivities of these peptides in RIA system are summarized in Fig. 2. Crossreactivity represents the ratio of the concentration of 1-34hPTH to those of 1-34hPTH analogues or 1-34bPTH where the concentrations are those required to displace 50% of 125I-labelled 1-34hPTH from antiserum. In this system, the cross reaction of NLeu$^8$-NLeu$^{18}$-1-34hPTH (3.9%) and that of 1-34bPTH (7.1%) were far less than that of NLeu$^8$-NLeu$^{18}$-1-34hPTH (20%).

(4) Crossreactivity of the antiserum to 1-84bPTH and 1-84hPTH;
As shown in Fig. 3, crossreactivity of R-I with 1-84bPTH and 1-84hPTH, was less than 1% in each case.

(5) Serum PTH levels in various diseases;
Serum PTH levels in the control group were below 0.6 ng/ml in 1-84PTH RIA and below 0.11 ng/ml in 1-34hPTH RIA, as shown in Fig. 4a, b. In the 1-84PTH RIA system, the percentage of normal adults with an undetectable level of serum 1-84PTH was 45%, while that of cases with a serum PTH level of 0.1-0.6 ng/ml was 55%. On the other hand, in the 1-34hPTH RIA system, all the normal subjects showed a serum level of 1-34hPTH below 0.11 ng/ml.

In patients with CRF, serum PTH levels in both assay systems were high, but the incidence of cases with an abnormally high level of serum PTH was higher in the 1-84PTH assay system (90%) than in the 1-34hPTH assay system (39%). In 4 cases of PHP, serum PTH levels were above the normal range in both assays. There were some patients whose serum PTH levels were abnormally high in senile osteoporosis (1-
Figure 3. Crossreactivity of R-I with 1-84 bTH and 1-84 hPTH. Crossreactivities of R-I with 1-34 hPTH (●), 1-84 bPTH (○) and 1-84 hPTH (△) are shown in this figure.

Discussion

To analyse the immunological properties and the clinical significance of the circulat-
ing PTH fragments, particularly those of 1–34hPTH, which is believed to be biologically active, in various disorders of calcium metabolism, we have developed a radioimmunoassay (RIA) which specifically measures 1–34hPTH and 1–84PTH.

To date, little information has been available on the immunological properties of the PTH fragments. Visser et al. (1979) reported that both C-terminal and N-terminal amino acids of 1–34hPTH were involved as immunological recognition sites in 1–34hPTH molecule. Segre et al. (1976) also reported that the C-terminal region of 1–34hPTH molecule was a significant immunological determinant site. According to our present data, it appeared that the amino acids at positions around 8 and 18 of 1–34hPTH molecule were also important as the immunological recognition site together with the C- and N-terminal regions as previously reported, since the conformational change in the position of 8 and 18 amino acids in the 1–34hPTH molecule resulted in approximately an 80% reduction in crossreactivity against native 1–34hPTH in 1–34hPTH RIA.

The conformational change in the position of 34 amino acid (NLeu8–NLeu18–Tyr34 1–34hPTH) resulted in further a decline in crossreactivity when compared with that of NLeu8–NLeu18–1–34hPTH in the antiserum R-I. 1–34bPTH showed weak crossactivities with the antiserum against 1–34hPTH.

These findings suggest that the antiserum R-I mainly recognizes the position of the C-, N-terminal region and amino acids at around 8 and 18 of the 1–34hPTH molecule.

As shown in Fig. 3, the cross reaction of 1–84hPTH with antiserum R-I was less than 1%. Therefore, we concluded that the antiserum R-I could measure circulating 1–34hPTH specifically.

It is possible that the clinical significance of measuring serum PTH levels by 1–84PTH RIA is different from that by 1–34PTH RIA. In the patients with CRF, the incidence of cases with high serum PTH level was 90% when measured by 1–84PTH RIA, but it was only 39% when measured by 1–34hPTH RIA. The high level of serum PTH in CRF may be caused either by hypersecretion of PTH (secondary hyperparathyroidism) (Roth and Marshall, 1969) or by impaired metabolism of PTH resulting in the retention of the COOH terminal fragment of PTH molecule (Lustenberger, 1978). In view of these data, it is possible that circulating serum PTH in CRF may consist of a relatively large amount of COOH terminal and/or intact molecule and a small amount of NH2 terminal fragment of PTH. It is of interest to study whether or not patients with abnormally high level of 1–34hPTH in serum show osteitis fibrosa cystica.

It has been reported that some patients with osteoporosis had a high level of serum PTH (Fujita et al., 1973; Teitelbaum et al., 1976; Riggs 1979; Orimo and Shiraki, 1979). However, it is still not clear whether the cause of the high PTH level in osteoporosis is due to hypersecretion of PTH or to the prolonged metabolism of circulating PTH. According to our present data, the incidence of cases with a high PTH level was equal when assayed with both 1–34hPTH RIA and 1–84PTH RIA, and the serum levels of PTH measured by those two RIA systems were well correlated. These data suggested that the high level of PTH seen in a part of senile osteoporosis may be due to hypersecretion of this hormone.

Serum PTH levels in PHP were abnormally high in all cases and those in HP were low in both assays.

These results clearly demonstrated that these two specific RIAs for PTH were useful in detecting the circulating PTH and sometimes help to distinguish the increase in the secretion of PTH from the retention of PTH fragments due to impaired metabolism of PTH.
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References


