NOTE

Radioimmunoassay of Parathyroid Hormone in Human Plasma and Tissue Using Commercially Available Antiserum and Modified Iodination

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Synopsis

The human PTH radioimmunoassay has been developed by use of bovine PTH and anti-bovine PTH antiserum, both of which were commercially available. The iodination process was modified as to use acid solution resulting in the more stable $^{125}$I-PTH. However, the PTH radioimmunoassay employed this antiserum with $^{125}$I-bovine PTH revealed that the N-terminal portion of PTH molecule was reactive only slightly in this system, indicating that most of PTH determined by this radioimmunoassay might be an biologically inactive PTH.

Plasma from normal subjects and patients with primary hyperparathyroidism or uremia showed dilutional curves parallel to the curve of the standard bovine PTH, indicating the immunological identity between human and bovine PTH in this radioimmunoassay system. The extract from the parathyroid adenoma showed an identical dilutional curve, but the extract from the ectopic PTH producing tumor showed a different dilutional curve from that of the standard PTH, which might indicate the immunochemical heterogeneity of PTH produced from the ectopic source. Plasma PTH levels obtained from normal subjects and patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism were compatible with those reported previously. These data indicate that the human PTH radioimmunoassay can be done by use of commercially available anti-bovine PTH antiserum and bovine PTH in conjunction with the modified iodination process and double antibody method. However, this assay was directed mainly to the C-terminal portion of PTH molecule and the PTH radioimmunoassay specific for the N-terminal portion will be required to evaluate the PTH regulation more precisely in man.

Since Berson et al. reported the radioimmunoassay of parathyroid hormone (PTH) in human plasma and tissue (Berson et al., 1963), there have appeared several papers concerning the human PTH radioimmunoassay (Reiss and Canterbury, 1968; Schopman et al., 1970; Arnaud et al., 1971; Segre et al., 1972; Hori et al., 1972; Reity and Weinstein, 1973). However, human PTH is hard to obtain even in small quantity to use for the iodination. Berson et al. used crude bovine PTH to produce antibodies in guinea pigs and the more purified bovine PTH for the iodination and the assay standard (Berson et al., 1963). Most papers concerning the human PTH radioimmunoassay were employing bovine PTH as antigen for both the assay standard and the immunization, but Arnaud et al. used anti-porcine PTH antiserum, bovine PTH for the iodination and plasma from a patient with primary hyperparathyroidism for the assay standard (Arnaud et al., 1971). Therefore, it was required to examine the specificity of each PTH radioimmunoassay system prior to determining plasma or tissue PTH levels in man.

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Furthermore, it has become recognized that PTH in human plasma is immunoheterogenous and more than one immunoreactive species of PTH circulate in plasma (Arnaud et al., 1972). Therefore, the PTH radioimmunoassay using different antiserum could produce different results.

These data indicate that each PTH radioimmunoassay system should be examined carefully what portion of PTH molecule is reacting with the antibody.

Recently, anti-bovine PTH antisera have become available commercially. By using this antiserum, we have developed the PTH radioimmunoassay in human plasma and tissue in conjunction with characterizing the specificity of the antiserum. In addition, we have modified the iodination process of PTH and increased the protein concentration of the incubation mixture in order to keep PTH stable and to avoid the adsorption on glass-wares. The double antibody method was employed to separate the bound from the free.

Here, we are reporting the human PTH radioimmunoassay using bovine PTH and anti-bovine PTH antiserum, both of which are commercially available, with the modified process of iodination.

**Materials and Methods**

Highly purified bovine PTH was obtained from Wilson Laboratories, Park Forest South, Ill., U.S.A. Synthetic \(^{1-34}\) bovine PTH was obtained from Beckman Instruments, Inc., Palo Alto, Ca., U.S.A. Anti-bovine PTH antisera (211/41 and 211/32) were purchased from Wellcome Research Laboratories, Beckenham, England. Bovine or human albumin was the fraction V powder commercially available. QUSO was obtained from Philadelphia Quartz Co., Chester, Pa., U.S.A. \(^{125}\) I was obtained from the New England Nuclear, Boston, Mass., U.S.A., as a carrier free Na\(^{125}\) I. PTH free plasma was collected from patients with hypercalcemia due to generalized bone metastases.

**Iodination of PTH**

The method of iodination was based on that reported by Hunter and Greenwood (Hunter and Greenwood, 1962), which was modified in the following way. To a conical plastic tube containing 1.5 \(\mu\)g bovine PTH (5 \(\mu\)l), 25 \(\mu\)l 0.4 M phosphate buffer, pH 7.5 and approximately 1 mCi Na\(^{125}\) I were added in this order. Twenty \(\mu\)l chloramine-T (1 mg/ml 0.04 M phosphate buffer, pH 7.5) was added twice with about 15 second interval. The mixing was accomplished by bubbling gently through the pipette used to add the chloramine-T solution. The total mixing time was about 30 seconds. Chloramine-T was always dissolved just prior to use. Succeedingly, 50 \(\mu\)l sodium metabisulfite (1 mg/ml distilled water) and 10 \(\mu\)l KI were added in turn and the throughout mixing was done after adding each solution. The mixture was then immediately transferred on Bio-Gel P-10 column, which was pretreated as follows. Bio-Gel P-10 suspended in 0.1 M acetic acid was packed in Excel column (SM 300, 0.9 x 20 cm) and saturated with 2 ml 0.1 M acetic acid containing 2% bovine albumin, which was followed by washing with 20 ml 0.1 M acetic acid. One tenth M acetic acid was used as eluent and the eluate was collected successively in tubes containing 0.05 ml 2% bovine albumin in 0.1 M acetic acid solution. Specific activity was calculated by adding 5 and 10 fold counts of the \(^{125}\) I-PTH to the PTH radioimmunoassay system.

**Preparation of incubation mixture**

The final incubation volume was 0.3 ml, which consisted of 0.1 ml PTH free plasma containing PTH standard or 0.1 ml assay sample, 0.1 ml \(^{125}\) I-PTH and 0.1 ml antiserum properly diluted with diluent buffer containing 1% normal guinea pig plasma. The diluent buffer was 0.08 M Veronal buffer, pH 8.6, containing 2% human albumin and 500 KIU/ml Trasylol. This diluent was used for diluting \(^{125}\) I-PTH. To prepare the standard PTH solution, plasma of a normal subject that had been absorbed with 2.5% QUSO was employed. This plasma was compared with that of a patient with hypercalcemia due to bone metastases and both were proved to behave identically in the PTH radioimmunoassay system.

The stock solution of bovine PTH (300 \(\mu\)g/ml 0.1 M acetic acid) was diluted to 10 \(\mu\)g/ml with 0.1 M acetic acid containing 0.5% bovine albumin. Each of 0.1 ml PTH solution was kept at \(-70^\circ\)C and one tube was employed to prepare the standard curve for each assay. At the time to set up the incubation mixture, 0.9 ml PTH free plasma was added to the tube (1 \(\mu\)g/ml) and the further dilution was carried out with the PTH free plasma. The counts of \(^{125}\) I-PTH added to each tube were about 3,000 cpm equivalent to approximately 25 pg PTH. Seven tubes containing only the diluted \(^{125}\) I-PTH were prepared.
to obtain the total counts.

The incubation was carried out for 4 days at 4°C and 0.1 ml anti-guinea pig γ-globulin antiserum diluted properly with the diluent buffer was added. After the further incubation for 2 days at 4°C, the tubes were centrifuged at 1,500 G for 20 min by cold centrifuge and the supernatants were discarded. The sediments were counted by a well type automatic γ-counter. The ratio of the bound to the total was calculated and the amount of PTH present was determined according to the usual process of the radioimmunoassay.

Extraction of PTH from tumor tissues

The extraction was performed based on the method reported by O'Riordan et al. (O'Riordan et al., 1971). The tissue stored at -20°C was weighed and dried by homogenizing on ice twice with acetone precooled to -20°C (2.5 ml per g of tissue), then twice with hexane (also precooled to -20°C, 2.5 ml per g), and again with the precooled acetone (2.5 ml per g). The dried powder was stirred in 8 M urea in 10% acetic acid (10 ml per g) for 2 hr at room temperature; 5 volumes of a solution of acetic acid-acetone 1:4, and 0.004 M sodium chloride were added, and the mixture was cooled at 4°C for 1 hr. The supernatant was separated by centrifuge and an equal volume of ether was added to the supernatant. After standing overnight at 4°C, the active precipitate was collected on a filter, washed with acetone, and dried in a vacuum. Sodium chloride was added to a concentration of 6%. The precipitate was removed by centrifuge. The supernatant was dialyzed against water for 2 days at 4°C and lyophilized. The lyophilized material was dissolved in the PTH radioimmunoassay diluent buffer containing 2% human albumin (1 ml per g). To apply the radioimmunoassay, the further dilution was carried out by the PTH free plasma. The recovery was examined by adding 125I-PTH or the standard PTH to the homogenized tissue, which yielded 15% and 48% respectively.

Results

Iodination of bovine PTH

Specific activity determined by the PTH radioimmunoassay system was at a range of 100–150 μCi/μg, indicating 1 iodine atom was attached to about 2 molecules of PTH.

The iodinated PTH was aliquoted and stored -20°C. After one month storage, the repurification of 125I-PTH through Bio-Gel P-10 column revealed that there is no significant damage or free 125I. Therefore, we were using 125I-PTH without repurification.

Standard curve

The standard curve using antiserum 211/41 diluted to 1: 90,000 showed that the trace B/T value was around 0.35, which was displaced by 5 ng bovine PTH to less than 0.05 (Fig. 1). Ten per cent fall level was observed at the standard PTH concentration of at least 0.015 ng in every assay, indicating that it was possible to detect plasma PTH concentration as low as 0.15 ng/ml. However, the standard curve using antiserum 211/32 diluted to 1: 160,000 was much less sensitive to that of 211/41 and it was possible to detect 0.5 ng of the standard PTH per tube.

Dose response and cross reaction

Dilutional curves of plasma from patients with uremia or primary hyperparathyroidism, tumor extracts from patients with parathyroid adenoma or the ectopic PTH syndrome, and synthetic 1–34 bovine PTH were depicted in Figure 1, when the antiserum 211/41 was employed. Plasma from patients with uremia or primary hyperparathyroidism and the extract from parathyroid adenoma showed the dilutional curve identical to that of the standard bovine PTH. However, the extract of the ectopic PTH producing tumor showed the different dilutional curve from that of the standard. Synthetic 1–34 bovine PTH showed a slight cross reaction in this system, but the dose response curve was quite different from that of the standard PTH.

Plasma PTH levels in patients with various diseases

Normal PTH levels measured by this radioimmunoassay were less than 0.5 ng/ml. Plasma PTH levels of patients with primary hyperparathyroidism were high at a range of 0.8–5.3 ng/ml. A patient with parathyroid carcinoma had a distinctly elevated plasma
Fig. 1 Dilutional curves of the standard bovine PTH, synthetic 1-34 bovine PTH, plasma from patients with primary hyperparathyroidism or uremia, and the tumor extracts from patients with parathyroid adenoma or pseudohyperparathyroidism, using antiserum 211/41 diluted to 1:90,000.

**Fig. 1** Dilutional curves of the standard bovine PTH, synthetic 1-34 bovine PTH, plasma from patients with primary hyperparathyroidism or uremia, and the tumor extracts from patients with parathyroid adenoma or pseudohyperparathyroidism, using antiserum 211/41 diluted to 1:90,000.

PTH level of 11.3 ng/ml. Plasma PTH levels of patients with uremia ranged from 0.8 to 29.0 ng/ml. In patients with hypercalcemia due to bone metastases and a patient with primary hypoparathyroidism, plasma PTH levels were undetectable and less than 0.15 ng/ml. In patients with hypercalcemia due to bone metastases and a patient with primary hypoparathyroidism, plasma PTH levels were undetectable and less than 0.15 ng/ml. In patients with the ectopic PTH syndrome (pseudohyperparathyroidism), plasma PTH levels were detectable in a range of 0.15-0.5 ng/ml even in the presence of marked hypercalcemia.

The extracts of parathyroid adenoma and carcinoma revealed the PTH content of 12.5 and 1.35 μg/g of wet tissue respectively. The extract of ectopic PTH producing tumor was found to have 27 ng PTH per gram of the wet tissue.

**Discussion**

It has been well recognized that plasma or tissue PTH levels are important to be determined in many clinical conditions, especially in patients with hyper- or hypocalcemia. However, the PTH radioimmunoassay is not so popular like the other hormones such as GH, Insulin, TSH, LH or FSH. The reason is that PTH is hard to obtain in enough amount for immunizing animals and highly purified PTH as used for the iodination was not available. However, it has become possible recently to obtain both anti-bovine PTH antisera and highly purified bovine PTH from the commercial sources. Using these materials, we have developed the radioimmunoassay of human PTH.

However, it has become recognized that human PTH is immunoheterogenous - that is, that more than one immunoreactive species of PTH are present (Berson and Yalow, 1968; Segre et al., 1972; Arnaud, 1973). These observations indicate that plasma PTH values obtained by the radioimmunoassay are determined by what portion of PTH molecule reacts with the antibody used for
the radioimmunoassay. Therefore, it is very important to decide that the antiserum employed for the radioimmunoassay reacts for what portion of PTH molecule, especially in the antiserum which is commercially available.

According to our data, synthetic $^{1-34}$bovine PTH showed a slight cross reaction in this system, indicating that most of PTH determined by this system is a material with the structure of C-terminal portion of PTH. These data indicate that most of PTH determined by this system might be biologically inactive, as reported by Arnaud et al. (Arnaud et al., 1972).

However, plasma from patients with primary hyperparathyroidism or uremia showed the dilutional curves identical to the curve of the standard bovine PTH, indicating an immunological identity between them. The values obtained from normal subjects and patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism were compatible with those reported previously (Potts, Jr. et al., 1971, Reitz and Weinstein, 1973). These data indicate that this system is sufficient to determine the PTH levels in human plasma and tissue in the ordinary sense of PTH radioimmunoassay.

It is interesting to note that the tumor extract from a patient with pseudohyperparathyroidism showed the different dilutional curve from that of the standard bovine PTH. These observation may indicate the immunological heterogeneity of PTH produced from the ectopic source, which might explain the fact that plasma PTH levels in patients with pseudohyperparathyroidism were usually lower than those obtained from patients with primary hyperparathyroidism (Riggs et al., 1971).

We have used acid solution to purify $^{125}$I-PTH following the iodination. This is based on the fact that PTH is stable and less absorbed on Bio-Gel or glasswares at acid pH. (Schopman et al., 1970). This modification provided us the more stable $^{125}$I-PTH which could be used without further purification. We used 2% protein concentration in the diluent buffer and plasma volume in the incubation mixture was increased to 1/3 of the total incubation volume in order to avoid the adsorption on glass-wares. In conjunction of the modified iodination process and higher protein concentration in the incubation mixture with the double antibody method to separate the bound from the free, it was possible to develop the sensitive radioimmunoassay of human PTH.

It can be concluded that the PTH radioimmunoassay in man can be done more easily by employing the method described herein. However, the PTH radioimmunoassay which is specific for the N-terminal portion of PTH molecule will be required to evaluate the PTH regulation more precisely in man.

References


