Radioimmunoassay of Serum Parathyroid Hormone in Postmenopausal Osteoporosis*

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Synopsis

Radioimmunoassay of parathyroid hormone (PTH) in human serum was carried out using guinea pig antibody against bovine parathyroid hormone and 125I labeled highly purified bovine PTH employing dextran-coated charcoal to separate the bound and free fraction. With the use of this method, PTH was detected in the serum of about 85% of 118 normal male and female subjects of various ages. PTH in serum was definitely elevated in primary and secondary hyperparathyroidism and pseudohypoparathyroidism but was undetectable or low in idiopathic and postoperative hypoparathyroidism. In 11 patients with postmenopausal osteoporosis with compression fracture, serum PTH was significantly higher than in age-matched controls. Serum PTH increased along with the progress in the severity of vertebral bone change in X-ray picture.

Materials and Methods

I. Radioimmunoassay of PTH

1) Antibodies

Partially purified bovine PTH (Wilson, 250 units/mg), 1–3 mg dissolved in physiological saline and emulsified with equal volume of complete Freund Adjuvant, was injected subcutaneously in guinea pigs every 2 weeks. After more than 5 injections, blood samples were obtained through cardiac puncture into heparinized syringe. Plasma was immediately separated and tested for the binding capacity of 125I-labeled PTH by the antibody at various dilutions with Veronal buffer, pH 8.6. The diluted antibody was preserved at 0°C with addition of 0.1% Merthiolate. At the dilution of antibody higher than 10,000, the capacity of binding 125I-PTH rapidly declined and no appreciable binding occurred at dilutions above 20,000.

2) 125I-labeled PTH

Highly purified PTH (Wilson, 3,000 USP units/mg) was labeled with 125I by the method of Hunter and Greenwood (1966) and purified through adsorption to microfine silica powder (Quix G32) (Yalow and Berson, 1966). Specific activity of 200–230 mCi/
mg was obtained. ¹²⁵I-labeled PTH was diluted with Veronal buffer, pH 8.6, with 0.5% bovine serum albumin and stored at -20°C. Purity of ¹²⁵I-labeled PTH and binding with antibody was demonstrated by chromatoelectrophoresis, paper-chromatography, adsorption to dextran coated charcoal, and gel filtration with Sephadex G-100. ¹²⁵I moiety which dose not combine with the antibody at antibody excess ("damage" fraction) occupied less than 10% of the ¹²⁵I in the ¹²⁵I-PTH preparation.

(3) Method of Radioimmunoassay

Serum samples, 0.1 ml of original serum and appropriate dilutions with hypoparathyroid serum, were incubated with 0.1 ml of antiserum diluted 5,000 times with Veronal buffer, pH 8.6, and 0.1 ml of Veronal buffer, pH 8.6, for 72 hr at 4°C. Solution of ¹²⁵I labeled PTH, 0.05 ml, was then added and the mixture was further incubated at 4°C for 48 hr. At the end of the incubation, 0.2 ml of the suspension of dextran coated charcoal was added and thoroughly mixed. The mixture was then immediately centrifuged for 3 min at 3,000 rpm and the supernatant was transferred to another test tube. ¹²⁵I radioactivity of the charcoal precipitate (f) and supernatant (b) was determined in an Aloka automatic scintillation counter. Another series of samples and standards to which 0.1 ml of Veronal buffer, pH 8.6, was added instead of antiserum were similarly processed and corresponding radioactivity obtained as (f') and (b'). B/F of the samples were calculated as

$$B/F = \frac{b - b'}{f} \text{ where } d = \frac{b'}{b' + f'}(b + f).$$

For each assay, tubes with antibody excess and antigen excess were set up along with those without antigen or without antibody. Protein content of each tube was kept constant through addition of serum obtained from hypoparathyroid patient.

Comparison of the dilution curves between standard uremic serum and bovine parathyroid hormone revealed an agreement at lower concentrations with some divergence at higher concentrations. It was therefore possible to express the amount of PTH in human serum in ng/ml bovine PTH equivalent.

(4) Blood sampling

Blood samples were obtained from 55 normal males ranging in age from 23 to 83 years and 63 females ranging in age from 21 to 81 years, at 9 a.m. after overnight fasting. These normal subjects were free of definite osteoporosis in lateral lumber spine X-ray pictures. Serum was immediately separated and kept frozen at -15°C until the time of the assay. Blood samples were similarly obtained and assayed from 29 patients with postmenopausal osteoporosis, 11 of them with compression fracture, 20 patients with primary hyperparathyroidism, 20 patients with secondary hyperparathyroidism due to renal insufficiency, 5 patients with postoperative hyperparathyroidism, 5 patients with idiopathic hyperparathyroidism and 4 patients with pseudohyperparathyroidism. Degree of vertebral deformity was expressed quantitatively by the method of Nordin et al. (1966).

Results

Figure 1 shows the dilution curves for bovine PTH and standard human serum PTH. Despite some discrepancy between these 2 curves at higher concentration, partial superimposition makes it possible to calculate human serum PTH in terms of ng/ml bovine PTH.

Table 1. Parathyroid hormone in serum of normal subjects of various ages

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of subjects</td>
<td>Number of undetectable samples</td>
</tr>
<tr>
<td>80 and over</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>70–79</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>60–69</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>50–59</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>40–49</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>30–39</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>20–29</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>9</td>
</tr>
</tbody>
</table>
Reproducibility of the Assay of Human Serum and Comparison with Bovine PTH

![Dilution curves for bovine PTH and standard human serum PTH. The results of human serum PTH assay represent the average and standard error of B/F in 7 assays. Partial superimposition is achieved and amount of PTH in standard human serum may be expressed in terms of ng/ml bovine PTH. Trace B/F is shown in shaded area, as average and standard error of 7 assays. PTH is considered to be detectable when B/F differs significantly from trace B/F.]

Table 1 summarizes the immunoreactive PTH level in normal human serum. PTH was undetectable or below 0.15 ng/ml in 9 of 55 males and 9 of 63 females. There appears to be a tendency of decrease of PTH in serum with advancing age in both males and females, though the variation was rather wide in each age group.

Table 2 summarizes the serum PTH level in primary hyperparathyroidism, secondary hyperparathyroidism, postoperative and idiopathic hypoparathyroidism, and pseudohypo-
Serum PTH in Postmenopausal Osteoporosis with Compression Fracture

Fig. 2. Serum parathyroid hormone expressed in bovine parathyroid hormone equivalent (ng/ml) in osteoporotics with compression fracture and age-matched controls without osteoporosis.

parathyroidism. Serum PTH was definitely elevated in primary and secondary hyperparathyroidism and pseudohypoparathyroidism but was undetectable in postoperative and idiopathic hypoparathyroidism.

Figure 2 shows the serum PTH in 11 patients with postmenopausal osteoporosis with compression fracture. Osteoporotics as a group had significantly higher serum PTH level than normal subjects of corresponding age, but 4 of the 11 of these patients had serum PTH level overlapping the normal range and 7 had definitely elevated levels. Figure 3 shows the significant negative correlation between serum PTH and lumbar score or the degree of lumbar spine deformity, suggesting a progressive rise of serum PTH as the spinal osteoporosis advances.

Discussion

Radioimmunoassay of PTH in human plasma has been carried out by various
Table 2. Parathyroid hormone in serum of patients with various parathyroid diseases

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
<th>Parathyroid hormone ng/ml ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary hyperparathyroidism</td>
<td>20</td>
<td>2.19 ± 0.24</td>
</tr>
<tr>
<td>Secondary hyperparathyroidism</td>
<td>20</td>
<td>2.65 ± 0.22</td>
</tr>
<tr>
<td>Postoperative hypoparathyroidism</td>
<td>5</td>
<td>all undetectable</td>
</tr>
<tr>
<td>Idiopathic hypoparathyroidism</td>
<td>5</td>
<td>all undetectable</td>
</tr>
<tr>
<td>Pseudohypoparathyroidism</td>
<td>4</td>
<td>1.50 ± 0.15</td>
</tr>
</tbody>
</table>

methods but serum from normal subjects was frequently undetectable except in the studies of Reiss et al. (1968). Arnaud et al. (1971) were able to detect PTH in 95% of normal subjects, using an antibody to porcine PTH, which is not yet widely available. Since it is mandatory to detect PTH in most of the normal subjects to study the change of serum PTH according to age and compare it with that in osteoporotics, we have attempted to produce antibodies against bovine PTH in guinea pig with high titer and good binding capacity to human PTH. The method of Schopman et al. (1970) was essentially followed because of its apparent simplicity and high sensitivity with utmost precautions in setting up damage control. This method proved rather satisfactory in our hands in precision and reproducibility, and was able to detect as little as 0.15 ng/ml bovine PTH equivalent.

Definitely elevated PTH levels in serum in primary and secondary hyperparathyroidism as well as pseudohypoparathyroidism and undetectable level of PTH in postoperative and idiopathic hypoparathyroidism might indicate the usefulness of this method of radioimmunoassay of PTH in the diagnosis of diseases of the parathyroids.

Changes of serum PTH according to age and sex have not been sufficiently documented. With the use of the present method, which is capable of detecting PTH in about 85% of normal subjects, no definite sex difference appeared to be present but a tendency of

![Serum PTH and Lumbar Score](image)

Fig. 3. Correlation between serum parathyroid hormone expressed in bovine parathyroid hormone equivalent and Lumbar Score or the ratio between the middle and anterior height of the third lumbar vertebra expressing the degree of deformity.
decline with advancing age was noted in serum PTH.

In postmenopausal osteoporosis with compression fracture serum PTH appeared to be definitely higher than in normal subjects of corresponding age without evident osteoporosis. Though the accurate assessment of the severity of osteoporosis in X-ray film is notoriously difficult, a progressive increase in the severity of osteoporosis is suggested in view of the significant negative correlation between lumbar score, an index of the progress of vertebral deforming due to osteoporosis and serum PTH level. Serum calcium and phosphorus stayed within normal range in all these patients with osteoporosis. Serum alkaline phosphatase was slightly elevated in 2 of 11 patients with compression fracture but normal in all others. No radiographic evidence suggesting osteomalacia or hyperparathyroidism was noted in any of these cases. Although no bone biopsy was carried out, it is most likely that these patients had primary postmenopausal osteoporosis and no concurrent osteomalacia or osteitis fibrosa.

Essential role of PTH in the development of osteoporosis was frequently suggested, since the increase of bone resorption was found to be predominant in osteoporosis. Parathyroidectomy prevented the development of experimental osteoporosis induced by low calcium diet and by immobilization (Burkhardt & Jowsey, 1967; Jowsey & Raisz, 1964). Arnaud et al. (1972) found increased PTH level in about 15% of their patients with postmenopausal osteoporosis and explained it with normocalcemic hyperparathyroidism occurring in a limited number of patients with osteoporosis. Development of postmenopausal osteoporosis was explained by the loss of protective effect of estrogen on the action of parathyroid hormone on bone (Nordin, 1971; Hossain et al., 1970) through demonstration of thinner metacarpal cortical thickness in hyperparathyroid patients compared to hypoparathyroid patients after the menopause but not before menopause. Pak et al. (1969) treated patients with osteoporosis with intravenous calcium infusion and obtained positive calcium balance, explaining the effect with suppression of parathyroid hormone secretion or stimulation of calcitonin secretion.

Rise of serum parathyroid hormone in patients with osteoporosis is apparently not a physiological age-dependent process, since a general tendency of decline in serum PTH is noted in subjects without evident osteoporosis. It is therefore tempting to suggest that osteoporosis is not a consequence of natural aging of the skeletal system but represents a limited group of patients with disturbance of calcium metabolism, with secondary rise of serum PTH. Decrease of calcium absorption from the intestine in patients with osteoporosis is of interest, in view of the possible significance as a trigger for such sequence of event (Cannigia et al., 1963; Bullamore et al., 1970).

References

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