NOTE

A Radioimmunoassay for Rat Serum Parathyroid Hormone using N-terminal of PTH

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Synopsis

Partially purified bovine parathyroid hormone (PTH) produced two kinds of antisera, GP-100 reacting with $^{125}$I-labeled bovine PTH (b-PTH 1–84) alone and GP-M reacting with $^{125}$I-labeled synthetic N-terminal portion of bovine PTH (b-PTH 1–34) as well as b-PTH 1–84. Rat serum PTH reacted with the latter but not the former, suggesting a similarity to b-PTH 1–34. Rat serum PTH became undetectable after parathyroidectomy, and increased after Na$_2$-EDTA injection and following renal injury with sodium sulfacetylthiazole.

Parathyroid hormone (PTH) in rat serum appears to be different in immunological behavior from PTH in rat parathyroid glands and culture medium, since a guinea pig antiserum to partially purified bovine PTH (GP-100) reacted with the latter but not the former (Fujita et al., 1974a; Okano et al., 1974). Since another guinea pig antiserum against partially purified bovine PTH reacting with the synthetic N-terminal portion of bovine PTH (b-PTH 1–34), has recently been obtained, attempts were made to conduct radioimmunoassay of rat serum PTH using GP-M.

Materials and Methods

Antiserum analysis

Partially purified bovine PTH (Wilson, 250 USP units/mg) dissolved in physiological saline was homogenized with equal amount of Freund’s complete adjuvant and administered in 10 guinea pigs subcutaneously with 2 week interval. Two of the potent antisera, GP-100 and GP-M, were used. Highly purified bovine PTH (Wilson, 971 adenyl cyclase units/mg) was labeled with $^{125}$I by the method of Hunter and Greenwood (1962), followed by purification with Quso G-32. The inhibition of the combination between $^{125}$I-PTH 1–84 and GP-100 or GP-M by various concentrations of b-PTH 1–84 and synthetic b-PTH 1–34 was tested through incubation at 4°C for 48 hours and separation of the bound and free fraction by dextran-coated charcoal. Both GP-100 and GP-M failed to react with other peptide hormones such as ACTH, TSH, insulin and HGH.

Radioimmunoassay of rat serum PTH

Synthetic b-PTH 1–34 (Beckman) was labeled with $^{125}$I by the method of Hunter and Greenwood (1962), followed by purification with Quso G-32. Rat serum samples, 0.1 ml and those diluted with thyroparathyroidectomized rat serum and b-PTH 1–34 standards were incubated with 0.1 ml 600×dilution of GP-M antiserum were incubated at 40°C for 72 hours and 0.1 ml $^{125}$I b-PTH 1–34 was added and the mixture was again incubated for 48 hours. Bound and free fractions were separated by addition of 0.2 ml dextran-coated charcoal suspension (2 g Norit-A and 0.3 g Dextran-80 suspended in 200 ml 0.05 M barbital sodium buffer, pH 8.6) and centrifuged at 3,000 r.p.m. for 3 minutes. After quickly removing the supernatant, radioactivity of the charcoal precipitate (FA) and supernatant (BA) was measured in an Aloka Automatic Scintillation Counter. $B/F$ was calculated as $B_A/D$, where $D = B_D(B_A + F_A)$.

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and $F_D$ and $B_D$ corresponded $F_A$ and $B_A$ respectively in a series without containing antiserum.

Changes of rat serum PTH

Twenty adult male rats of about 250 g body weight were maintained on Oriental Rat Chow containing 1.76% calcium, 1.04% phosphorus and 100 IU of vitamin D/100 g and water ad libitum. Intraperitoneal injection of 0.003 mole/Kg disodium ethylenediamine tetraacetate (Na$_2$-EDTA) was carried out in 5 rats and blood samples were obtained from the jugular vein 30 minutes later. Parathyroidectomy by cautery was carried out in 5 rats under ether anesthesia and blood samples were obtained 3 hours later. Na-sulfacetylthiazole as 5% aqueous solution was intraperitoneally injected twice a week for 4 weeks at a dose of 0.2 g/Kg and blood samples were obtained 24 hours after the last injection. The last group of 5 rats served as the control. Serum was separated by centrifugation at 3,000 r.p.m. for 10 minutes and radioimmunoassay was carried out as described above. Marked elevation of serum urea N and inorganic phosphorus was noted after Na-sulfacetylthiazole treatment, as reported previously (Okano et al., 1972).

Results

As shown in Fig. 1, b-PTH 1–84 inhibited the combination between GP-100 or GP-M and $^{125}$I-b-PTH 1–84 equally as expected, while b-PTH (1–34) inhibited the combination between GP-M and $^{125}$I-b-PTH 1–84 much better than GP-100 and $^{125}$I-b-PTH 1–84, suggesting a special affinity of GP-M to b-PTH (1–34). By the use of $^{125}$I-b-PTH 1–34 and GP-M in a radioimmunoassay system, rat serum PTH gave a dilution curve.

![Fig. 1. Inhibition of combination between GP-100 (cross) or GP-M (closed circle) and $^{125}$I-b-PTH (1–84) by b-PTH (1–84) (dotted line) and b-PTH (1–34) (solid line). In the abscissa, contents of b-PTH (1–84) (lower) and b-PTH (1–34) (upper) in the tube are shown, while B/F ratio (%) is shown on the ordinate. While b-PTH (1–84) markedly suppressed the combination between b-PTH (1–84) and GP-100 or GP-M, b-PTH (1–34) suppressed the combination between GP-M and $^{125}$I-PTH (1–84) considerably better than that between GP-100 and $^{125}$I-PTH (1–84).]
indistinguishable from that of b-PTH 1-34 (Fig. 2). Comparison with the use of b-PTH (1-34) gave the values given in Table 1. After parathyroidectomy, PTH in rat serum became undetectable, while serum PTH markedly rose after Na$_2$-EDTA or renal injury with Na-sulfacetylthiazole.

**Discussion**

The present method represent the first successful attempt of measuring rat serum PTH by an antiserum reacting with the N-terminal peptide of bovine PTH. Hargis et al. (1974) reported a method of radioimmunoassay of PTH in rat serum.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number of Animals</th>
<th>PTH ng/ml ± S.E.M.</th>
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<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Parathyroidectomized</td>
<td>5</td>
<td>undetectable</td>
</tr>
<tr>
<td>EDTA-injected</td>
<td>5</td>
<td>10.3±0.2</td>
</tr>
<tr>
<td>SAT*-injected</td>
<td>5</td>
<td>7.4±0.2</td>
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*S Sodium sulfacetylthiazole.

Fig. 2. Dilution curve of standard synthetic b-PTH (1-34) and rat serum, in the radioimmunoassay system using GP-M and $^{125}$I-b-PTH (1-34). The abscissa indicates concentrations of b-PTH (1-34) standard (lower) and rat serum PTH (upper). The ordinate shows % B/F.
using chicken anti-bovine PTH antiserum (CH 698), giving a value of 7.0 ± 1.5 pg/ml in normal rat, undetectable levels after parathyroidectomy, and a marked rise after EDTA infusion, low calcium diet and nephrectomy. The present result obtained by using guinea pig antiserum to partially purified bovine PTH reacting with synthetic N-terminal peptide of bovine PTH (b-PTH 1–34) and ¹²⁵I-labeled b-PTH 1–34 gave much higher values. The low values obtained by Hargis et al. (1974) might be explained by the high susceptibility to degradation and resulting extremely short half life of b-PTH (1–84)-like PTH in rat serum (Fujita et al. 1974b). In fact, PTH was undetectable in our hand as long as a guinea pig antiserum to b-PTH (G-100) poorly reacting with b-PTH 1–34 was used, although PTH in rat parathyroid glands and culture medium was readily measured by this antiserum (Okano et al., 1974; Fujita et al., 1974a). Blood sampling from the jugular, femoral or tail vein caused no change in the results. The present method therefore appears to be more suitable for measuring rat serum PTH especially the stable component resembling b-PTH (1–34) in its immunological property. Heterogeneity of PTH in tissue and blood has been repeatedly pointed out since Berson and Yalow (1968). The mode of secretion and degradation of PTH appears to vary in different animal species.

The present method of measurement of rat serum PTH would contribute to the understanding of the mechanism of PTH secretion and degradation in rat.

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References