Homologous Radioimmunoassay for Human Parathyrin (Residues 53–84)

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We describe a sequential saturation double-antibody radioimmunoassay for carboxyl-terminal fragments of human parathyrin (hPTH) in serum. Standards are prepared with synthetic hPTH (residues 53–84) in hPTH-free serum. Antisera are obtained by immunizing guinea pigs with partly purified hPTH extracted from adenomatous glands. Tracer is prepared by labeling hPTH (53–84), presumably at the histidine residue, with 125I by the Chloramine T method at pH 8.6. Dilution curves for hPTH extracted from adenomas are superimposable on dilution curves for the synthetic 53–84 fragment. Dilution of sera from hyperparathyroid patients showed linearity of response with concentration in the present assay, but non-linearity in the heterologous radioimmunoassay. In contrast to the heterologous system, which discriminated 28 of 32 patients with primary hyperparathyroidism from 32 normals (normal range: undetectable to 54 pmol/L, omitting the highest and lowest values from controls), the present assay separated these groups without overlap.

Additional Keyphrases: hormones • reference interval • radiolodination • equivalence of results for human parathyrin and a commercially available synthetic fragment

Parathyrin (PTH) is secreted by the parathyroid glands in response to changes in the concentration of calcium in the serum. The gland mainly secretes intact PTH (residues 1–84) and some fragments. In the circulation, intact PTH is rapidly degraded, the main organs for extraction being kidney and liver (1). Whereas the biological half-life of the intact hormone is very short, less than 5 min, the half-life of circulating carboxyl-terminal fragments is 40–60 min (2). The carboxyl-terminal fragments are mainly extracted from plasma by the kidney via glomerular filtration and degradation in the tubules (2).

Measurement of carboxyl-terminal fragments helps distinguish patients with primary hyperparathyroidism from healthy controls (3). In renal failure, the greatly increased concentration of circulating carboxyl-terminal fragments results from hypersecretion of the hormone by the parathyroid glands and its inadequate removal from the circulation. The main disadvantage of existing carboxyl-terminal PTH assays is that sometimes results for healthy individuals and patients with proven primary hyperparathyroidism overlap. This overlapping could be related to (e.g.) differences in the structure of bovine, porcine, and human hormone, resulting in incomplete recognition of the structure of the human hormone by the antibody. Therefore, we have tried to develop a largely homologous radioimmunoassay system for measurement of PTH in human serum, based on the use of a commercially available synthetic fragment of human parathyrin (residues 53–84).

Materials and Methods

Reagents and standards. Mix 800 mL of 67 mmol/L Na2HPO4 (no. 6587) with 200 mL of 67 mmol/L KH2PO4 (no. 4875; both from Merck AG, Darmstadt, F.R.G.) to give a pH 7.4 buffer. To this buffer add 1 g of human serum albumin (Behringwerke AG, Marburg, F.R.G.), 500 mg of thimerosal (Serva AG, Heidelberg, F.R.G.), and 400 mg of EDTA (Merck AG), to prepare buffer A. Antisera are obtained by immunizing guinea pigs with partly purified hPTH, extracted from adenomas, similar to the procedure of Bouillon et al. (4) (see below). Dilute 200 μL of antiserum with 750 mL of buffer A and 250 mL of aprotinin (Trasylol; Bayer, Leverkusen, F.R.G.), to give a concentration of 5 kalikrein inactivator mega-units/L. Add 5.8 mL of normal guinea-pig serum to this antiserum solution, resulting in a total of 6.0 mL of guinea pig serum per liter of antiserum solution.

Assay standards. Human plasma was rotary-mixed with charcoal (1 L of serum with 120 g of Norit A from Serva) overnight at room temperature. The mixture was then centrifuged (Sorvall RC2-B centrifuge, 5000 rpm, 1 h, rotor GS 3). The supernatant serum was filtered through filter no. 311651 (Schleicher & Schüll, Dassel, F.R.G.), then centrifuged overnight at 6000 rpm and 4 °C. The supernate was again filtered through the same material. Bovine serum was treated in the same way.

C-Terminal hPTH (53–84) fragment was from Paesel, Frankfurt-am-Main, F.R.G.

Goat anti-guinea-pig gamma-globulin was purchased from Deutsche Wellcome GmbH, Burgwedel, F.R.G. (cat. no. RD 18).

To obtain material for eliciting guinea-pig anti-PTH antibody, we homogenized 100 g of human parathyroid adenoma tissue in 400 mL of glycine hydrochloride (pH 3.0, 0.1 mol/L), centrifuged it in a Sorvall RC-2B for 1 h, and re-extracted the sediment with the glycine buffer. We collected 10-mL fractions and measured their absorbance at 280 nm (for protein content) and their PTH content by a heterologous immunoassay according to Bouillon et al. (4). We then injected about 10 to 20 μg of this partly purified hPTH six times, at monthly intervals, into guinea pigs. The antisera used in these experiments was that...
which produced the most sensitive standard curve (serum from guinea pig 7).

Iodination. We radiolabeled 5 μg of hPTH (53–84) with 1 mCi of Na125I (Amersham Buchler, Frankfurt-am-Main, F.R.G.) by a modification of the Hunter and Greenwood procedure (5), as follows.

Combine 5 μg of hPTH (53–84) in 10 μL of the pH 7.4 phosphate buffer, 20 μL (1 mCi) of Na125I in 20 μL of another phosphate buffer (0.5 mol/L, pH 9.0), and 10 μL (10 μg) of Chloramine T. Let this mixture react for 30 min at room temperature, stop the reaction by adding 50 μL (125 μg) of sodium metabisulfite, and then add 200 μL (2 mg) of Blue Dextran (Mf 200 000; Pharmacia). Apply the iodination mixture to a 30 × 0.9 cm Sephadex G25 column and elute with the pH 3.0 glycine buffer. Collect 1-mL fractions (about 15 drops each). Count the radioactivity in 10 μL of each fraction for 0.1 min in a Searle 1285 triplicate gamma counter (Zinsser, Frankfurt-am-Main, F.R.G.).

The first peak of radioactivity, which co-eluted with Blue Dextran, was diluted to 40 × 10^3 cpm/100 μL with buffer A and, if not used promptly, was stored frozen at −30 °C. The specific activities were about 60 to 80 Ci/g. These tracers were stable for at least eight weeks.

Radioimmunoassay. We routinely assayed each standard-curve concentration and each sample in triplicate. Individual nonspecific count measurements were checked in one assay, but this step was later omitted because all samples exhibited the same low nonspecific binding. The incubation mixture—100 μL of standard or patient’s sample, 100 μL of buffer A, and 100 μL of first antibody—was incubated in "Radioimmunoassay-special vials" (W. Sarstedt, Nürnberg, F.R.G.). After preincubation with the antisera for 24, 48, 72, or 240 h, 100 μL of 125I-labeled hPTH (53–84) containing about 40 × 10^3 cpm (1300 Bq) was added, and the incubation was continued for another 48 h. Then 100 μL of the second antibody was added and incubated for 6 h. Thereafter the tubes were centrifuged (10 min, 2000 × g) to separate bound peptide from free. The supernate was aspirated and discarded, and the sediment was washed again with 500 μL of buffer A. The tubes were again centrifuged for 10 min at 2000 × g and the supernates again aspirated and discarded. The radioactivity of the sediment was counted until 10 000 counts were accumulated. The nonspecific counts (average of one triplicate in each assay) were subtracted from the average of each triplicate determination of sample and standard. The bound radioactivity (linear scale, y-axis) was graphed vs the concentration of the competitor [hPTH (53–84) or adenoma extract, logarithmic scale, x-axis].

Serum calcium was determined routinely with an SMA 12/60 (Technicon Instruments Corp., Tarrytown, NY 10591).

Results

Standards

We prepared hPTH (53–84) standards in human plasma or bovine serum, both previously stripped with charcoal (Norit A). Then hPTH (53–84) was added in a concentration of 2000 pmol/L. This standard was serially diluted (1000, 500, 250, 125, 62, and 31 pmol/L) and used to prepare two different sets of standard curves, one with the standard in human plasma and the other in bovine serum. Euparathyroid controls and hyperparathyroid samples were measured as described below. The results (not shown) demonstrated that charcoal-treated bovine serum is superior for our purposes because hypoparathyroid patients yielded counting rates near that of the zero standard. In contrast, charcoal-treated human plasma contained immunoreactive material, and hypoparathyroid

![Graph](https://via.placeholder.com/150)

**Fig. 1.** Standard curves for (left) hPTH (53–84) standards and tracer and (right) bPTH (1–84) standards and tracer

Human parathyroid gland extract in several dilutions (•); (○) hPTH (53–84) and bPTH (1–84) standards.
Therefore, we performed all further experiments with charcoal-treated bovine serum.

Two further standard-curve experiments were performed: (a) with hPTH (53-84) diluted in charcoal-treated bovine serum, and (b) with bovine parathyrin (1-84) (bPTH, donated by Prof. O'Riordan, Middlesex Hospital, London, U.K.) diluted in charcoal-treated bovine serum. In addition to labeling hPTH (53-84), for the following experiment we also labeled bPTH (1-84) by the Chloramine T method at pH 7.4 according to Hunter and Greenwood (5): 2 μg of bPTH (1-84), 10 μg of Chloramine T, oxidation for 30 s.

In experiment a, hPTH (53-84) standard was incubated with the antiserum for 48 h, and 125I-labeled hPTH (53-84) was added as tracer and again incubated for 48 h at 4 °C.

In experiment b, bPTH (1-84) standard was incubated with the antiserum, and 125I-labeled bPTH (1-84) was added as tracer under the same conditions. The result of the experiment is shown in Figure 1.

As can be seen from Figure 1, extracted human parathyrin is as competitive as hPTH (53-84)—the lines are nearly parallel; such is not the case with bPTH (1-84) (Figure 2). Therefore, we concluded that use of hPTH (53-84) as standard and tracer could improve the assay for carboxyl-terminal fragments of human parathyrin (hPTH) in serum, as compared with heterologous assays involving 125I-labeled bPTH (1-84) as tracer.

**Analytical Variables**

**Sensitivity.** The most sensitive standard curve is produced by use of 100 μL of standard and 48 h of preincubation. Fifty percent displacement is at 270 pmol/L with this procedure, in contrast to 680 pmol/L when 50 μL of standard and 24 h of preincubation are used (Figure 2). The results of these and other experimental conditions are shown in Table 1.

**Specificity.** Synthetic hPTH (1-84) yielded no displacement of 125I-labeled hPTH (53-84). Human parathyroid gland extract displaces hPTH (53-84) tracer in parallel to hPTH (53-84). Therefore we concluded that only the carboxyl-terminal portion of hPTH is recognized in this assay system.

**Linearity.** Hyperparathyroid serum from a patient with primary hyperparathyroidism was diluted serially with charcoal-treated bovine serum. Undiluted hyperparathyroid serum and different serum dilutions were measured in the hPTH (53-84) and the bPTH (1-84) assay systems. The results are shown in Figure 3.

Figure 4 shows that both assay systems yield nearly linear results at serum dilutions. However, the concentration of parathyrin determined with the hPTH (53-84) assay system is about fivefold that with the bPTH (1-84) assay system. Figure 2 shows nonlinearity in the bPTH (1-84) assay if human parathyroid extract is determined at different dilutions.

**Clinical Use**

We evaluated healthy persons, healthy persons after calcium infusion, and patients with primary hyperparathyroid-
Table 2. Serum Calcium and hPTH (53–84) before, during, and after Calcium Infusion (10 mg Ca^{2+}/kg)*

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Serum calcium, mmol/L</th>
<th>hPTH (53–84), pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.13</td>
<td>69</td>
</tr>
<tr>
<td>70</td>
<td>2.62</td>
<td>41</td>
</tr>
<tr>
<td>145</td>
<td>3.24</td>
<td>&lt;30</td>
</tr>
<tr>
<td>220</td>
<td>3.28</td>
<td>&lt;30</td>
</tr>
<tr>
<td>300</td>
<td>3.05</td>
<td>37</td>
</tr>
</tbody>
</table>

*a Calcium was infused from min 1 to min 220

Discussion

Hitherto, parathyrin in human serum has been determined mainly by radioimmunoassays in which 125I-labeled bPTH is used as tracer (4, 7–12, 17). We have claimed the necessity for a homologous radioimmunoassay for human parathyrin because, with the various heterologous radioimmunoassays, hyperparathyroid patients were not distinguished from healthy controls (13). Use of standards of hPTH extracted from human adenomatous glands, antiserum against this extracted human parathyryn, and 125I-labeled bPTH (1–84) improved the radioimmunoassay for hPTH in serum (10). As we have shown, synthetic hPTH (53–84) may also be used as the standard, and these standard curves are superimposable on curves for various dilutions of human parathyryn extracted from adenomas.

Antisera against hPTH can be produced by immunizing guinea pigs with partly purified hPTH (1–84) or (work in progress) by immunizing rabbits with synthetic hPTH (53–84) complexed to thyroglobulin according to Sofroniew et al. (14). Relatively little 125I is incorporated into hPTH (53–84) as compared with other peptides. This may be caused by the absence of tyrosine and the presence of only one histidine in this fragment. Shortening the oxidation time yielded tracer of low specific activities. Use of the lactoperoxidase labeling technique (15) resulted in insufficient incorporation of 125I (results not shown). High sensitivity was only achieved by two or three days of preincubation followed by addition of tracer, similar to the incubation procedure by Di Bella et al. (8) and Mallette (9). The concentrations of hPTH in the serum of healthy controls and patients with primary hyperparathyroidism demonstrated that the homologous radioimmunoassay for hPTH (53–84) is superior to the heterologous radioimmunoassay: more healthy controls are identified as such and there is less overlap with results for hyperparathyroid patients. The question has to be resolved whether a homologous assay with hPTH (53–84) or hPTH (1–84) is the more useful in the diagnosis of primary hyperparathyroidism (16). Further improvement of the assay may be achieved by attaching a tyrosine to the hPTH (53–84) molecule for improved radioiodination.

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References

10. Wood, W. G., Butz, R., Casaretto, M., et al., Preliminary results...


