Radioimmunoassay Specific for Amino (N) and Carboxyl (C) Terminal Portion of Parathyroid Hormone

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Synopsis

A radioimmunoassay specific for the amino (N) terminal portion of the parathyroid hormone (PTH) molecule (N-PTH radioimmunoassay) has been developed by iodinating synthetic 1-34bovine PTH (1-34bPTH) and using commercially available bPTH antiserum. A radioimmunoassay specific for the carboxyl (C) terminal (C-PTH radioimmunoassay) has been carried out by adding enough amount of 1-34bPTH to the PTH radioimmunoassay system. The data obtained from N- and C-PTH radioimmunoassay were compared with those obtained from the PTH radioimmunoassay. It was observed that plasma levels of N-PTH, indicating biologically active PTH, were only one 8th to 32th to those of PTH and those of C-PTH were almost equal to those of PTH. These data corresponded well with those reported previously by using the antiserum specific for each terminal of the PTH molecule from the other laboratory. The half life of plasma N-PTH and C-PTH determined following the removal of parathyroid adenoma was less than 10 min and about 45 min respectively. These data indicate that the N-PTH radioimmunoassay can be done by iodinating 1-34bPTH and using commercially available antiserum.

In 1968 Berson and Yalow reported that plasma parathyroid hormone (PTH) levels determined by the radioimmunoassay were different when the different antiserum was employed, which was the first observation about immunoheterogeneity of PTH in human plasma (Berson and Yalow, 1968). Recently, Silverman and Yalow reported that the difference had been due to the antiserum used and that one had been the antiserum specific for the amino (N) terminal and the other for the carboxyl (C) terminal portion of the PTH molecule (Silverman and Yalow, 1973).

During this period many papers had been reported from several laboratories about immunoheterogeneity of PTH (Arnoud et al., 1971; Habner et al., 1971; Canterbury et al., 1972; Segre et al., 1972; Goldsmith et al., 1973; Arnaud et al., 1973) and it has become recognized that the usual antisera used for PTH radioimmunoassay were specific mostly for the C-terminal and it had to wait a chance to obtain the antiserum specific for the N-terminal.

However, we have developed the PTH radioimmunoassay specific for the N-terminal by iodinating synthetic 1-34bovine PTH (1-34bPTH) and using commercially available antiserum which contains antibodies for both N- and C-terminal portion of PTH.
In addition, the PTH radioimmunoassay which could not react with 1-34 portion of the PTH molecule has been performed by adding enough amount of 1-34bPTH to the incubation medium to saturate antibodies against 1-34 portion of PTH. The data obtained from these assays were compared with those from the PTH radioimmunoassay.

Materials and Methods

Synthetic 1-34 bovine PTH (Lot No. 15012) was obtained from Beckman Instruments Inc., 1117 California Ave., Palo Alto, Ca. 94304, U.S.A. and bovine PTH from Wilson Pharmaceutical and Chemical Corporation, Chicago, Ill. U.S.A. PTH antisera were purchased from Wellcome Reagents Limited, Beckenham, England. 125I was obtained from New England Nuclear Corp., Boston, Mass. U.S.A. as a carrier free Na 125I.

Iodination of 1-34 bPTH

The iodination was based on the method reported by Hunter and Greenwood (Hunter and Greenwood, 1962), which was modified as follows. To a conical plastic tube containing 1.5 µg 1-34bPTH dissolved in 0.1 M acetic acid solution (5 µl), 25 µl 0.4 M phosphate buffer, pH 7.5 and approximately 1 mCi Na 125I were added in this order. The following process to use 0.1 M acetic acid for elution was the same as reported previously for the iodination for PTH (Tanaka et al., 1974).

Preparation of incubation mixture

The final incubation volume was 0.3 ml, which consisted of 0.1 ml PTH free plasma containing the standard PTH or 0.1 ml assay sample, 0.1 ml the iodinated PTH solution and 0.1 ml antiserum diluted properly 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 preparation was the one used for the PTH radioimmunoassay as reported previously from our laboratory (Tanaka et al., 1974) and the following modifications were employed for the radioimmunoassay specific for 1-34 portion of PTH molecule (N-PTH) and that for the other portion of PTH (C-PTH).

N-PTH radioimmunoassay

Instead of the iodinated bPTH, 125I-1-34bPTH properly diluted with the diluent buffer was used. The antiserum was 211/32 obtained from Wellcome and the final dilution was 1: 60,000.

C-PTH radioimmunoassay

This was based on the method reported by Segre et al. (Segre et al., 1972). 125I-bPTH was employed as was the case of the PTH radioimmunoassay. The PTH antiserum (211/41) was diluted to 1 : 20,000 with the diluent buffer which contained 50 ng/ml 1-28bPTH and 1% normal guinea pig plasma, and 0.1 ml was added to the incubation mixture.

The incubation was carried out for 4 days at 4°C and the double antibody method was used to separate the bound from the free. Extraction from the parathyroid adenoma was performed as previously reported (Tanaka et al., 1974).

Patients studied

They were 6 patients with primary hyperparathyroidism, 7 with uremia and 4 with pseudohypoparathyroidism. The diagnosis of primary hyperparathyroidism was done by the operation. All 4 patients with pseudohypoparathyroidism had typical clinical features and none responded to PTH administered exogenously. In 2 patients with primary hyperparathyroidism plasmas were obtained prior to and repeatedly following the removal of parathyroid adenoma in order to determine the half life of N- and C-PTH in human circulation.

Results

Iodination of 1-34 bPTH:

An example of the iodinated 1-34bPTH was depicted in Fig. 1. Specific activity determined by the N-PTH radioimmunoassay was around 70 µCi/µg, indicating one iodine atom was attached to about 8 molecules of 1-34bPTH.

Antiserum titration curve:

Two-fold dilution of the antiserum 211/32 was made serially up to 1 : 240,000 and 125I-1-34bPTH solution 4 mµCi or 125I-1-34 bPTH 4 mµCi plus cold 1-34bPTH 100 µg were added to determine the optimal antisem dilution. As shown in Fig. 2, the largest difference of B/T was observed at the antiserum dilution of 1 : 60,000, which was employed for the further assays.
Standard curve and dilutional curves of bPTH, extract of parathyroid adenoma or plasmas from patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism in N-PTH radioimmunoassay:

As shown in Fig. 3, the standard $^{1-34}$bPTH showed 10 per cent fall of B/T at dose of 0.01 to 0.03 ng/tube in every assay. A dilutional curve of bPTH was parallel to that of $^{1-34}$bPTH, but the degree of reactivity of bPTH was 3.4 times less to that of $^{1-34}$bPTH, according to an average of 8 assays.

Extract of parathyroid adenoma or plasmas from these patients showed a dilutional curve parallel to that of $^{1-34}$bPTH, except that plasma from patients with pseudohypoparathyroidism showed no reaction, indicating less than 0.1 ng $^{1-34}$bPTH Eq/ml of plasma.

| Standard curve and dilutional curves of $^{1-34}$bPTH, extract of parathyroid adenoma or plasmas from patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism in C-PTH radioimmunoassay: |

As shown in Fig. 4, $^{1-34}$bPTH did not show any cross-reactivity at all up to 10 ng/tube. However, the standard bPTH was observed to decrease B/T more than 10 per cent at dose of 0.01 to 0.03 ng/tube in every
assay. Extract of parathyroid adenoma or plasmas obtained from these patients showed a parallel dose response curve to that of the standard bPTH.

Fig. 4. C-PTH radioimmunoassay. Dilutional curves of bPTH, 1-34 bPTH were depicted with those of plasmas from patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism and extract of parathyroid adenoma.

Plasma N-PTH, C-PTH and PTH levels in patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism:

Plasma N-PTH and C-PTH levels determined by the methods described here were shown in Table 1 with plasma PTH levels obtained by the PTH radioimmunoassay. Plasma N-PTH levels were always lower than plasma C-PTH or PTH levels at a difference of one 4th to 16th, but plasma C-PTH levels were almost in the same range to plasma PTH levels. In parathyroidal effluent samples plasma levels of N-PTH were almost twice to those of C-PTH or PTH.

Plasma N- and C-PTH levels following the removal of parathyroid adenoma:

Plasma N- and C-PTH levels were determined prior to and repeatedly following the removal of parathyroid adenoma in 2 patients with primary hyperparathyroidism. As shown in Fig. 5, plasma N-PTH levels (0.8 and 1.9 ng bPTH Eq/ml) were decreased following the adenoma removal and became undetectable within 10 min. In contrast, plasma C-PTH levels (2.6 and 4.6 ng bPTH Eq/ml) were also decreased after the operation, but disappearance curves were much slower. These data indicated that the half life of N-PTH in human circulation was less than 10 min and that of C-PTH 40 and 50 min.

Assay variabilities:
Intra-assay and inter-assay variability of each PTH radioimmunoassay were shown in Table 2 and all of these were in reasonable ranges.

Table 1. Plasma N-PTH, C-PTH and PTH levels in patients with primary hyperparathyroidism, uremia and pseudohypoparathyroidism

<table>
<thead>
<tr>
<th>Disease</th>
<th>N-PTH ng bPTH Eq/ml</th>
<th>C-PTH ng bPTH Eq/ml</th>
<th>PTH ng bPTH Eq/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>primary</td>
<td>0.8</td>
<td>3.6</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>4.8</td>
<td>4.5</td>
</tr>
<tr>
<td>hyperparathyroidism</td>
<td>0.4</td>
<td>4.6</td>
<td>5.3</td>
</tr>
<tr>
<td>(parathyroidal effluent plasma)</td>
<td>0.8</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>25.0</td>
<td>9.5</td>
<td>11.0</td>
<td></td>
</tr>
<tr>
<td>7.6</td>
<td>3.2</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>uremia</td>
<td>0.6</td>
<td>2.2</td>
<td>2.6</td>
</tr>
<tr>
<td>0.7</td>
<td>3.0</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>13.0</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>2.9</td>
<td>25.0</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td>pseudohypoparathyroidism</td>
<td>&lt;0.3</td>
<td>2.3</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>&lt;0.3</td>
<td>2.6</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>&lt;0.3</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>&lt;0.3</td>
<td>0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

In order to compare the data obtained from 3 different radioimmunoassays, bPTH was used as the standard.
Table 2. Intra-assay and interassay variability of each PTH radioimmunoassay

<table>
<thead>
<tr>
<th>Assay</th>
<th>Intra-assay</th>
<th>Interassay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±S.D. (ng/mL)</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>N-PTH</td>
<td>2.9±0.3</td>
<td>10%</td>
</tr>
<tr>
<td>C-PTH</td>
<td>2.9±0.2</td>
<td>7%</td>
</tr>
<tr>
<td>PTH</td>
<td>1.2±0.2</td>
<td>18%</td>
</tr>
</tbody>
</table>

Fig. 5. Plasma N- and C-PTH levels following the removal of parathyroid adenoma. In 2 patients with primary hyperparathyroidism blood was obtained prior to and repeatedly following the removal of parathyroid adenoma in order to determine plasma N- and C-PTH levels. Closed circle indicates plasma N-PTH level and cross plasma C-PTH level.

Discussion

Since Berson and Yalow reported immunoheterogeneity of PTH in human plasma (Berson and Yalow, 1968), the PTH radioimmunoassay in which an antigenic determinant portion locates at a biological active part of PTH has been looked for. It has been known that biological activity of PTH locates at its N-terminal portion (Potts, Jr., 1973) and we have developed a sensitive and specific radioimmunoassay for the N-terminal by iodinating 1–34bPTH. 1–34bPTH is known to contain no tyrosine in its amino-acid sequence, but the iodination process done by the chloramine-T method brought an iodinated 1–34bPTH which was sufficient to be used for the 1–34PTH radioimmunoassay.

As reported previously from our laboratory (Tanaka et al., 1974), the PTH antisera provided from Wellcome contained antibodies for both bPTH and 1–34bPTH, indicating that these antisera could be used for the 1–34PTH radioimmunoassay with the iodinated 1–34bPTH. However, the amount of antibodies reacted with N-PTH was much less when compared with that of antibodies reacted with C-PTH. Therefore, we had to use much lower antiserum dilution for N-PTH radioimmunoassay.

Extract of parathyroid adenoma or plasmas from patients with primary hyperparathyroidism or uremia showed a dilutional curve parallel to that of 1–34bPTH, indicating immunological identity between them. Although a parallelism existed between 1–34bPTH and bPTH, the degree of reactivity of bPTH was 4.3 times less to that of 1–34bPTH. This ratio should be 2.3 when these materials were compared on molar basis, which might indicate that about one half of the bPTH used here lacks the N-terminal reactivity.

C-PTH radioimmunoassay was developed according to the method reported by Segre et al. (Segre et al., 1972). 1–34bPTH added to the PTH radioimmunoassay system blocked the reaction of 1–34bPTH up to 10 ng/tube, indicating that plasma 1–34PTH...
levels should be over 100 ng/ml to be detected as C-PTH in this system, but, the standard bPTH was possible to be detected down to 0.3 ng/ml. Extract of parathyroid adenoma or plasmas from patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism showed a parallel dose response curve to that of the standard bPTH. These observations indicate that PTH whose antigenic determinant portion does not locate within 1-34 portion of PTH molecule can be determined by use of this radioimmunoassay system. By employing both N-PTH and C-PTH radioimmunoassay in conjunction with the PTH radioimmunoassay, plasma PTHs levels in patients with primary hyperparathyroidism, uremia or pseudohypoparathyroidism were measured. It was found that plasma N-PTH levels were much lower than plasma C-PTH or PTH levels and that plasma N-PTH levels were only one 4th to 16th of plasma C-PTH, when bPTH was used as the standard. As one half of the bPTH used here lacks the N-terminal activity, this ratio would be one 8th to 32th, suggesting that about 90% of peripheral plasma PTH levels determined by the PTH radioimmunoassay could be biologically inactive. Plasma levels of C-PTH were in the same range to those of PTH, suggesting that most of PTH determined by our PTH radioimmunoassay were PTH whose antigenic determinant portion located at the part other than 1-34 portion of PTH molecule. These observations coincide with those reported by Segre et al. (Segre et al., 1972).

It is interesting to note that the parathyroid effluent bloods obtained from patients with parathyroid adenoma showed higher levels of plasma N-PTH than those of C-PTH or PTH. The ratios N-PTH to C-PTH or PTH were 2.4 and 1.7. However, when based on the fact that about half of the bPTH standard lacks the N-terminal reactivity, these levels could turn out to be about equal, which suggests that PTH with its complete structure was secreting from parathyroid adenoma.

It was showed that the half life of plasma N-PTH was apparently shorter than that of C-PTH, which may explain the fact that plasma levels of N-PTH were always lower when compared with those of C-PTH.

It should mentioned here that plasma N-PTH levels were undetectable in patients with pseudohypoparathyroidism, even though plasma C-PTH or PTH levels were elevated. These observations indicated that plasma PTH of these patients might be immunologically different at 1-34 portion of PTH molecule. However, plasma N-PTH levels were one 4th to 16th to those of plasma PTH levels and when considered plasma PTH levels in these patients, plasma N-PTH levels would be down in an undetectable range. Therefore, more studies are required to conclude that plasma PTH in these patients lacks N-terminal immunoreactivity and is biologically inactive.

However, the fact that the N-PTH radioimmunoassay could be developed by using the method described here suggests the possibility to determine the biological active PTH, which could be a big tool to investigate PTH regulation in man.

References


Goldsmith, R. S., J. Furszyfer, W. J. Jonson, A. E. Fournier, G. W. Sizemore and C. D.


