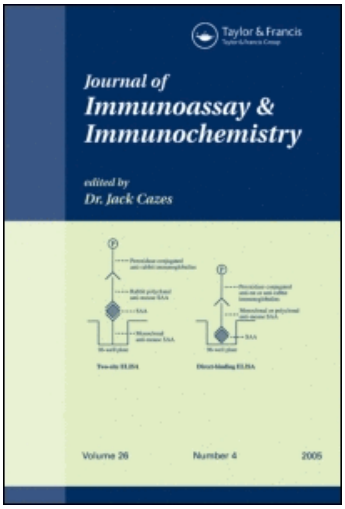

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Comparison of Four Different Carboxylterminal Tracers in a Radioimmunoassay Specific to the 68-84 Region of Human Parathyroid Hormone

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COMPARISON OF FOUR DIFFERENT CARBOXYLTERMINAL TRACERS IN A RADIOIMMUNOASSAY SPECIFIC TO THE 68-84 REGION OF HUMAN PARATHYROID HORMONE

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ABSTRACT

Two synthetic carboxylterminal fragments, {tyr^{52}}hPTH(52-84) and {tyr^{63}}hPTH(63-84), and purified bPTH(1-84) were iodinated with ^{125}Iodine to be compared as tracers in a late carboxylterminal radioimmunoassay. Tracer ^{125}I-bPTH(41-84) was generated *in vitro* by incubating ^{125}I-bPTH(1-84) with plasma membranes of rat kidney cortex. Region specificity was achieved by saturating the unwanted middle component of our multivalent antiserum with a molar excess of hPTH(44-68). A charcoal-dextran separation was worked out for each tracer. The titer of the antiserum giving ≅30% specific binding of each tracer was used in all experiments. Displacement of each tracer with increasing molar concentration of hPTH(1-84), hPTH(53-84), hPTH(41-84) and of hPTH (64-84) was studied. hPTH(41-84) was also generated by incubating hPTH(1-84) with rat cortex kidney membranes and was calibrated against a commercial preparation of bPTH(37-84). A progressive increase in the titer of the antiserum was seen as the molecular weight of the tracers decreased from a titer of 1/20,000 with ^{125}I-bPTH(1-84) to a titer of 1/50,000 with the two synthetic tracers. Similarly the so-called damage seen during the charcoal-dextran separation in absence of antibody was reduced from 16.0±6.2% (mean ±SD) with ^{125}I-bPTH(1-84) to 1.3±.2 with the two synthetic tracers. 50% displacement of the ^{125}I-bPTH(1-84) tracer was achieved at 13.2±.8 fmol/tube for hPTH(1-84) and at 6.3±1.0 fmol/tube for hPTH(41-84), reflecting the greater reactivity of fragments in that system. With the two synthetic tracers, a concentration of 5.0±.4 fmol/tube of hPTH(1-84) or of 3.5±1.2 fmol/tube of hPTH(41-84) was necessary to achieve the same goal. With ^{125}I-bPTH(41-84) results were between the two extremes. These results indicated that an increase in antiserum titer, a decrease in assay damage, an improvement in assay sensitivity and in comparative molar reactivity of the various circulating forms of hPTH can be achieved by using synthetic carboxylterminal fragments as tracers in region specific radioimmunoassays of hPTH.

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INTRODUCTION

The radioimmunoassay of human parathyroid hormone (hPTH) has been complicated by a variety of problems. Among the most difficult, one notes the heterogeneity of the product to be measured in blood (1) and the varying ability of each antiserum to recognize and react with the various circulating molecular forms of the hormone (2,3,4,5). This last phenomenon was better demonstrated during the characterisation of various antisera raised against partially purified bovine (b) or hPTH (6,7,8,9), when ^{125}I-bPTH(1-84) was used as tracer and intact hormone or synthetic fragments representative of the different structural regions of the b or hPTH molecules as standards. Most antisera were found to be multivalent, having two or more antigenic recognition sites, each one with a different affinity for the tracer, intact hormone or its synthetic or natural fragments (6,7,8,9). The different characteristics of each antiserum probably better explains the heterogeneity of the results obtained with the various hPTH radioimmunoassays (10,11). In order to simplify this type of problem, a number of attempts have been made to render multivalent antisera monovalent; this approach would, theoretically, permit a better understanding of the reaction, in a radioimmunoassay, of a given structural region of the b or hPTH molecule present in the intact extracted (1-84) form of the hormone or in circulating and/or synthetic fragments. This has been accomplished by raising antibodies against synthetic fragments representative of the region of the PTH molecule to be measured (12,13), by saturating the unwanted component(s) of a multivalent antiserum with an excess of synthetic peptide (6), or by limiting the reactivity of a multivalent antiserum through the use of a

synthetic tracer representative of the region to be measured (14,15,16). These different approaches have led to the concept of the region-specific assay. The role of the tracer in this type of assay has never been studied; it is not known whether small synthetic fragments would make better tracers than intact ^{125}I-bPTH(1-84). We have thus considered the behavior of four different carboxylterminal tracers, intact ^{125}I-bPTH(1-84), ^{125}I-bPTH(41-84), ^{125}I-{tyr^{52}}hPTH(52-84) and ^{125}I{tyr^{63}}hPTH(63-84), in a radioimmunoassay specific to the 68-84 region of the b or hPTH structure. This paper summarizes our results and indicates that there are various advantages to using small synthetic fragments as tracers.

MATERIALS AND METHODS

b and hPTH Preparations

bPTH(1-84) purified to the carboxymethylcellulose stage (17) was used for iodination. Synthetic {tyr^{52}}hPTH(52-84) and {tyr^{63}}hPTH(63-84) were purchased from Bacham (P.O. Box 3426, Torrance, Ca 90510) for the same purpose.

bPTH(1-84) standard 71/324 was provided by the National Institute for Biological Standards (Holly Hill, Hampstead, London, NW36RB, England). Synthetic bPTH(1-34) was purchased from Beckman (Palo Alto, Ca 94304) while synthetic bPTH(53-84) was kindly provided by Dr. M. Rosenblatt. Natural bPTH(37-84) was purchased from Immuno Nuclear Corporation (P.O. Box 285, Stillwater, Minnesota 55082).

hPTH(1-84) was obtained by extraction of pooled human parathyroid adenoma, and purified to the stage of gel filtration on BioGel P-100 (BioRad Laboratories) (9); this preparation was calibrated against hPTH(1-84) standard 75/549 (National Institute of Biological Standards)

in the carboxylterminal radioimmunoassay later described under «characterization of antiserum C-52». hPTH(1-34) and hPTH(53-84) were purchased from Peninsula Inc. (611 Taylor Way, Belmont, Ca 54002); hPTH(44-68) and hPTH(64-84) from Bacham.

Antiserum

Antiserum C-52 was produced in a guinea pig against partially purified bPTH. Details of the immunization program, and partial characterization of this antiserum have been published previously (9).

Serum from Patients

Sera from a patient with surgically proven primary hyperparathyroidism and one with tertiary hyperparathyroidism were used for these studies.

Methods

Iodination and Purification of the Tracers

bPTH(1-84), {tyr^{52}}hPTH(52-84) and {tyr^{63}}hPTH(63-84) were iodinated with ^{125}INa (New England Nuclear) by modification (6) of Hunter and Greenwood's method (18). Each tracer was further purified by gel chromatography on a 1.5 x 75 cm column of BioGel P-100. The peak tubes corresponding to the position of elution of each tracer were used in the assay.

Production of ^{125}I-bPTH(41-84) and of hPTH(...41-84)

^{125}I-bPTH(41-84) was produced by incubating ^{125}I-bPTH(1-84) with plasma membranes of rat kidney cortex. Membranes were prepared as previously described (19) except for ultracentrifugation on a continuous sucrose gradient, which was omitted. The last pellet ob-

tained by centrifugation was resuspended in citrate buffer 0.025 M, pH 4 to a concentration of 1 µg protein/µl of buffer. After iodination of 10 µg of bPTH(1-84) with 10 mCi of ^{125}INa, the reaction mixture (≅100 µl) was diluted to 500 µl by addition of 400 µl of ammonium acetate buffer 0.15 M, pH 4.6, 1% with bovine serum albumin. This solution was incubated 15 min. at 37°C with 500 µl (500 µg of membrane proteins) of partially purified plasma membranes of rat kidney cortex in citrate buffer 0.025 M, pH 4. Reaction was stopped by addition of 1 ml of cold citrate buffer and centrifugation to remove the membranes (3000 g x 20 min). The supernatant was purified by gel chromatography on BioGel P100. The radioactivity peak corresponding to the large carboxylterminal fragment(s) was further analyzed by Edman degradations to recover the ^{125}I-tyrosil residue present in position 43 of the bPTH structure in order to identify the cleavage position(s) (20). This was done as described previously (21).

hPTH(...41-84) was produced by incubating 30 µg of hPTH(1-84) with the same membranes in a similar protocol. The exact position of cleavage was not identified, and the end product was called hPTH(...41-84) by analogy with results obtained under similar circumstances with ^{125}I- bPTH(1-84). This preparation was characterized by its position of elution on BioGel P-100 and by its immunoreactivity in a carboxylterminal radioimmunoassay. It was calibrated to have a molar reactivity, when compared to hPTH(1-84), similar to the one seen in the carboxylterminal assay used to characterize antiserum C-52 for natural bPTH(37-84), when compared to bPTH(1-84).

Characterization of Antiserum C-52

The antiserum was studied with ^{125}I-bPTH(1-84) as tracer, at a 1/40,000 dilution giving nearly 30% binding. All components were

diluted in 0.05 M barbital buffer, pH 8.6, 10% with hypoparathyroid dog serum. Final volume was 500 µl. The assay was performed in non-equilibrium with 24-hour preincubation of the antiserum and standard prior to addition of the tracer, and 24-hour incubation after its addition. All work was carried out at 4°C. A charcoal-dextran method was used to separate the bound and free tracer. Displacement of the tracer with increasing molar concentrations of the various b of hPTH standards, alone or in combination, was studied.

Charcoal-Dextran Separation

A different charcoal-dextran separation of the bound and free tracer had to be worked out for each tracer. The conditions giving nearly 30% specific binding of each tracer had to be found by trial and error. In each case, 1 ml of a solution containing an increasing concentration of charcoal-dextran (10:1) in barbital buffer was added to tubes containing only the antiserum and the tracer, in order to find the conditions giving the highest specific binding with the least damage.

Comparison of the Various Tracers in a Late Carboxylterminal Radioimmnoassay

The assay was performed with each tracer as described under characterization of antiserum C-52. The antiserum was used after an excess of hPTH(44-68) had seen added to saturate the mid-carboxyl-terminal component; 20 ng/tube were used for this purpose. This made the antiserum specific for the 68-84 region of the b or hPTH structure. The titer giving nearly 30% binding of each tracer was used. Displacement of the tracer with increasing molar concentration of hPTH(1-84), hPTH(...41-84), hPTH(1-34), hPTH(44-68), hPTH(53-84) and hPTH(64-84) was studied in all cases. Furthermore, the concentration

of hPTH present in the serum of our two patients was estimated by assaying 10 consecutive dilutions (50, 25, 12.5 µl...) against hPTH(1-84). In all cases, the curves obtained were parallel to the standard. The molar ratio of hPTH(1-84)/hPTH(...41-84) for equal displacement of each tracer was estimated from the mean of 3 ratios obtained at 20, 50 and 80% displacement of the tracer.

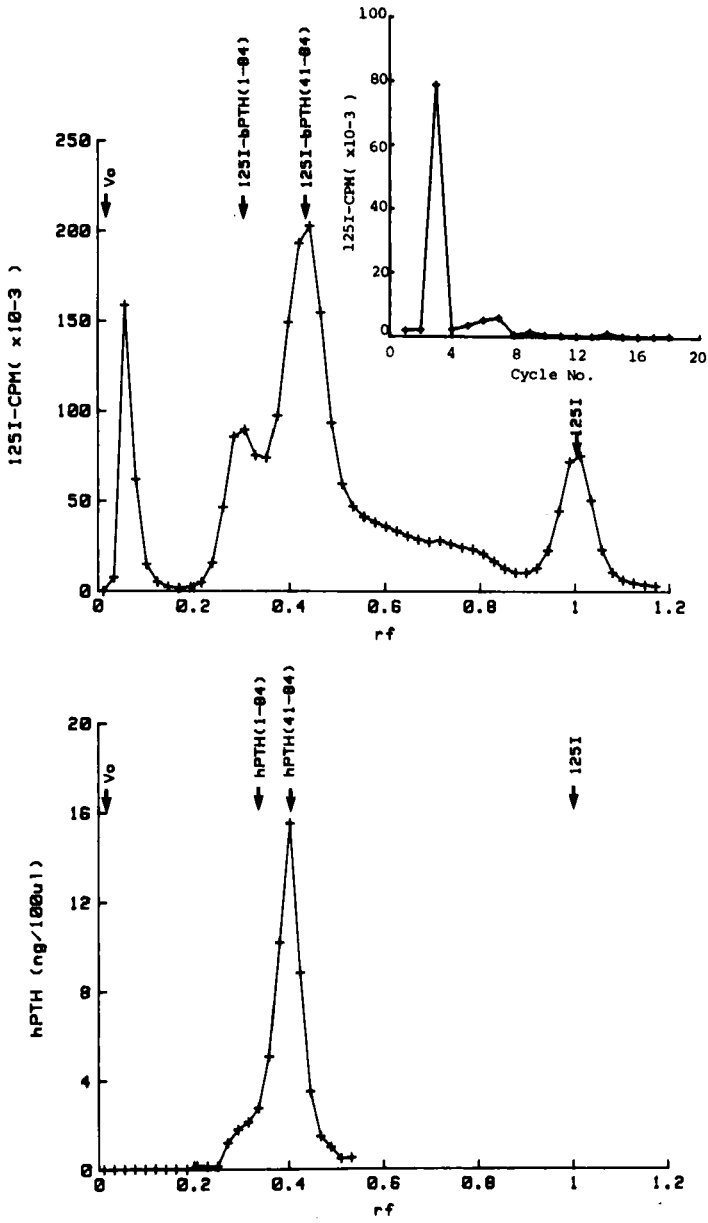
Statistical Analysis

Standard methods were used to calculate mean and standard deviation. A one way analysis of variance was used to compare the results of the various assays.

RESULTS

Production of ^{125}I-bPTH(41-84) and of hPTH(...41-84)

The characteristics of the carboxylterminal fragments produced by incubating ^{125}I-bPTH(1-84) and hPTH(1-84) with plasma membranes of rat kidney cortex are illustrated in Figure 1. The gel chromatography profile of the supernatant obtained after incubation of ^{125}I-bPTH(1-84) is illustrated at top; the one obtained after incubation of hPTH(1-84) below. In the first case, apart from the void and salt volume radioactivity, a certain amount of intact ^{125}I-bPTH(1-84) and a major peak of large carboxylterminal fragment(s) can be identified. Smaller fragments are also evident between the large fragment and the salt volume. The peak corresponding to the large carboxylterminal fragment was analyzed by sequence analysis to recover the ^{125}I-tyrosyl residue as shown in the inset. More than 90% of the radioactivity was recovered at cycle 3 indicating a position of cleavage between the position 40 and 41 of the bPTH structure. ^{125}I- bPTH(41-84) was thus



the main carboxyl terminal fragment obtained. The gel chromatography profile obtained with hPTH(1-84) is analyzed with the late carboxylterminal assay to be described in detail and ^{125}I-{tyr^{52}}hPTH (52-84) as tracer. Profile analysis disclosed a small amount of intact hormone and a large peak of a big carboxylterminal fragment. This peak migrated similarly to ^{125}I-bPTH(41-84) on BioGel P-100. Furthermore, this fragment completely displaced the ^{125}I-bPTH(1-84) tracer in our mixed carboxylterminal assay (Figure 2), thus indicating that the two antigenic sites recognized by that antiserum, regions 44-53 and 68-84, were present within its structure. The fragment was called hPTH(...41-84) by analogy with its bovine counterpart, although its real structure remains to be proven.

Characterization of Antiserum C-52

Displacement of the ^{125}I-bPTH(1-84) tracer bound to antiserum C-52 by increasing molar concentrations of various h and bPTH preparations is illustrated in Figure 2. h and bPTH(1-34) did not displace the tracer, reflecting the carboxylterminal nature of the antiserum. bPTH (37-84) could displace the tracer completely and was about twice as potent on a molar basis as bPTH(1-84). bPTH (53-84) could only

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FIGURE 1 ^{125}I-bPTH(41-84), and hPTH(...41-84) were produced by incubating ^{125}I-bPTH(1-84) and hPTH(1-84) with plasma membranes of rat kidney cortex. Details of the procedure will be found under Methods. Analysis of the incubation products was done by gel filtration on BioGel P-100. Profiles of an experiment with ^{125}I-bPTH (1-84) (top) and hPTH(1-84) (bottom) are illustrated. hPTH profile was analyzed with the 68-84 assay, using ^{125}I-{tyr^{52}}hPTH(52-84) as tracer and hPTH(1-84) as standard. The peak of radioactivity corresponding to carboxyl terminal fragment(s) was submitted to sequence analysis to identify the ^{125}I-tyrosyl residue present in position 43 of the bPTH structure. Three cycles were necessary to reach 90% of the ^{125}I-tyrosyl radioactivity indicating that the main fragment produced was ^{125}I-bPTH(41-84). The main fragment produced with hPTH(1-84) was considered, by analogy, to be hPTH(...41-84) but its exact structure remains to be proven.

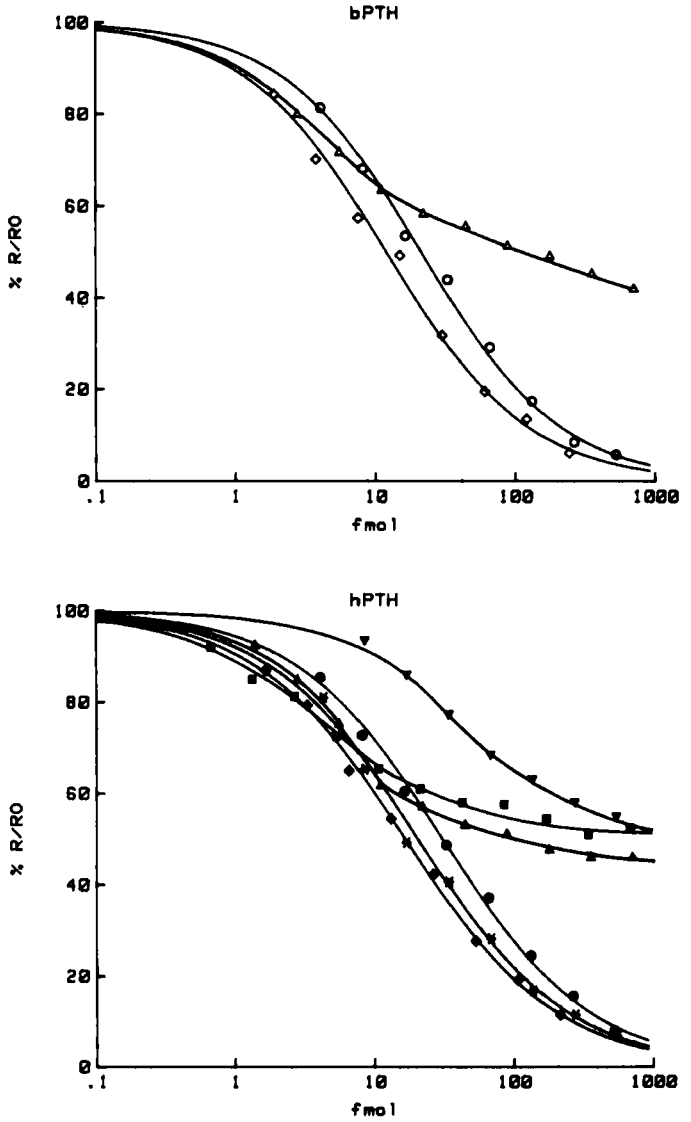

FIGURE 2 The characteristics of antiserum C-52 are illustrated through displacement of ^{125}I-bPTH(1-84) tracer by increasing molar concentration of b(○) or h(●) PTH(1-84), b(◇) PTH(37-84) or h(◆) PTH(...41-84), b(△) or h(▲) PTH(53-84), h(■) PTH(64-84), h(▼) PTH(44-68), hPTH(44-68) + hPTH(53-84) (*). Antiserum titer is 1/30,000: Bo/T is 37%.

displace 55% of the tracer, indicating two antigenic determinants in our antiserum, one in region 37-53 and the other in region 53-84. This was better illustrated with the various hPTH preparations. hPTH(1-84) and hPTH(...41-84) could displace the tracer completely; hPTH(...41-84) was calibrated in this assay to give a molar displacement, when compared to hPTH(1-84), similar to the one seen with bPTH(37-84) when compared to bPTH(1-84). hPTH(53-84) and hPTH(64-84) could only displace 55% of the tracer while hPTH(44-68) could displace the other 45% of the counts. Addition of hPTH(44-68) to hPTH(53-84) or hPTH(64-84) (not illustrated) resulted in complete displacement of the tracer. This confirmed the existence of two antigenic carboxylterminal components in our antiserum, the first being before position 53 and the second after position 68.

Charcoal-Dextran Separation

Figure 3 illustrates the results of the experiments that led to the choice of an appropriate charcoal-dextran concentration for the separation of each bound and free tracer. The titer of the antiserum giving 30% binding of each tracer was used. The conditions giving maximum specific binding with minimum damage were used in all cases. The amount of charcoal-dextran necessary for separation increased as the molecular weight of each tracer decreased, being .125 gm/dl for ^{125}I-bPTH(1-84), .5 gm/dl for ^{125}I-{tyr^{52}}hPTH(41-84), 1 gm/dl for ^{125}I-{tyr^{52}}hPTH(52-84) and 3 gm/dl for ^{125}I-{tyr^{63}}hPTH(63-84).

Comparison of Four Tracers in a 68-84 Assay of hPTH

These results are illustrated in Figure 4 and in Tables I and II. Figure 4 shows the displacement of each tracer by an increasing molar

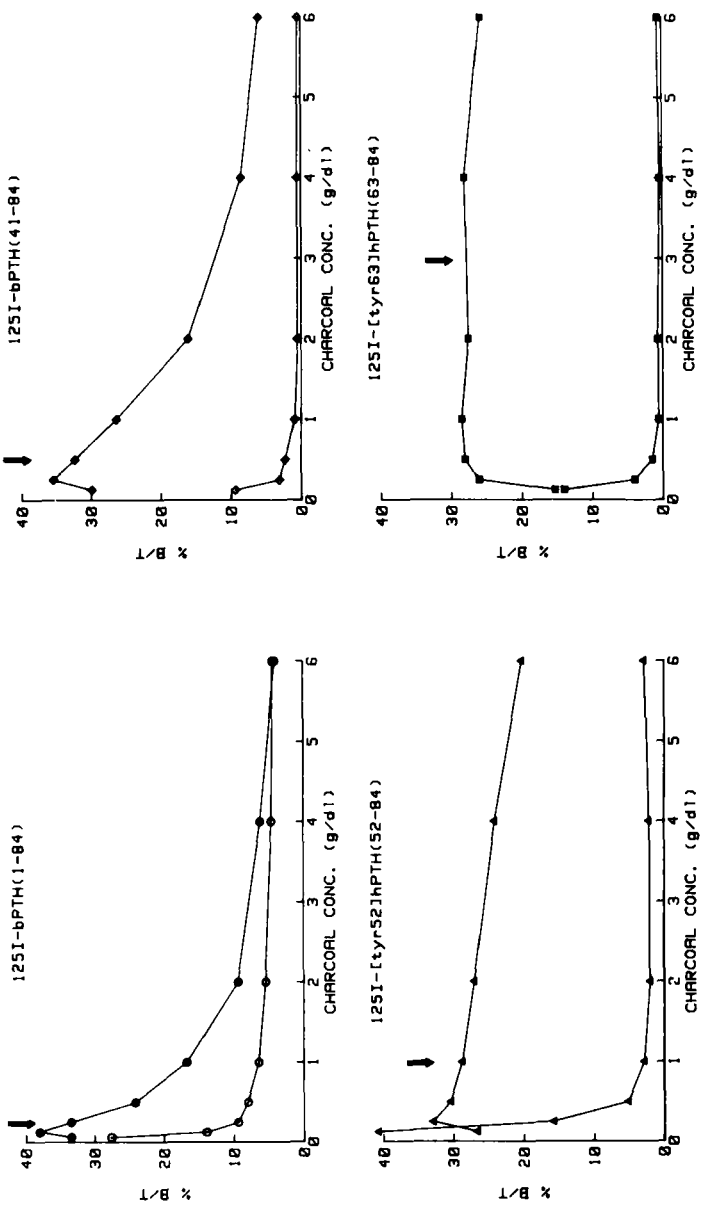

FIGURE 3 A different charcoal-dextran separation of bound and free tracer was worked out for each tracer. The evolution of damage (or the amount of tracer bound in the absence of antiserum) (clear symbols), and of specific binding (or total binding minus damage) (black symbols) is illustrated as a function of the charcoal-dextran concentrations used. In all cases, the concentration of dextran was 1/10 that of charcoal. The final concentration of charcoal used for each tracer is indicated by an arrow. As the molecular weight of each tracer decreased, more charcoal was necessary to achieve separation.

TABLE 1*

Characteristics of Each Region-Specific Radioimmunoassay

Characteristics / Tracers	Antiserum C-52	Bo/T (%)	.D (%)	fmol of hPTH(1-84) giving % Displacement 20%	50%	80%	fmol of hPTH(41-84) giving % Displacement 20%	50%	80%	hPTH(1-84)/hPTH(41-84) Molar Ratio for Equal Displacement
^{125}I-bPTH(1-84)	1/20,000	31.8 ±6.2	16.0 ± 6.2	5.0 ±.2	13.2 ± .8	78.6 ±6.4	1.9 ±.3	6.3 ±1.0	44.4 ±11.5	2.17 ±.43
^{125}I-bPTH(41-84)	1/30,000	32.4 ±4.4	2.6 ± .6	2.3 ±.3	8.9 ±1.5	32.1 ±2.7	1.3 ±.2	5.0 ± .2	21.2 ±1.7	1.69 ±.15
^{125}I-{tyr^{52}}hPTH(52-84)	1/50,000	29.0 ±2.9	1.6 ±1.5	1.3 ±.2	4.9 ± .2	19.0 ±1.2	.8 ±.1	3.4 ± .3	16.5 ± 3.0	1.41 ±.24
^{125}I-{tyr^{63}}hPTH(63-84)	1/50,000	27.8 ±1.0	.8 ± .5	1.3 ±.1	5.0 ± .4	19.4 ±2.8	.9 ±.4	3.5 ±1.2	15.0 ± 4.5	1.39 ±.08

* Results are expressed as mean ±SD of a minimum of two different assays. .D = damaged or % of tracer bound in absence of antiserum.

TABLE 2

Estimated Potency of Two Different Samples in Various Region Specific Radioimmunoassays

Tracers	Sample I (Primary Hyperparathyroidism) (fmol/ml)	Sample 2 (Renal Failure) (fmol/ml)
^{125}I-bPTH(1-84)	2427 ± 158^{o}	1718 ± 112^{o}
^{125}I-bPTH(41-84)	2440 ± 277	1599 ± 259
^{125}I-{tyr^{52}}hPTH(52-84)	1941 ± 102^{o}*†	1262 ± 0^{o}*†
^{125}I-{tyr^{63}}hPTH(63-84)	2072 ± 101	1307 ± 64*

Values are expressed as mean ±SD of 2 to 3^{o} different assays. Assay conditions as in Table I. Results obtained were compared by a one way analysis of variance (different from ^{125}I-bPTH(1-84), *; different from ^{125}I-bPTH(41-84), †; $p<.05$, * or †).

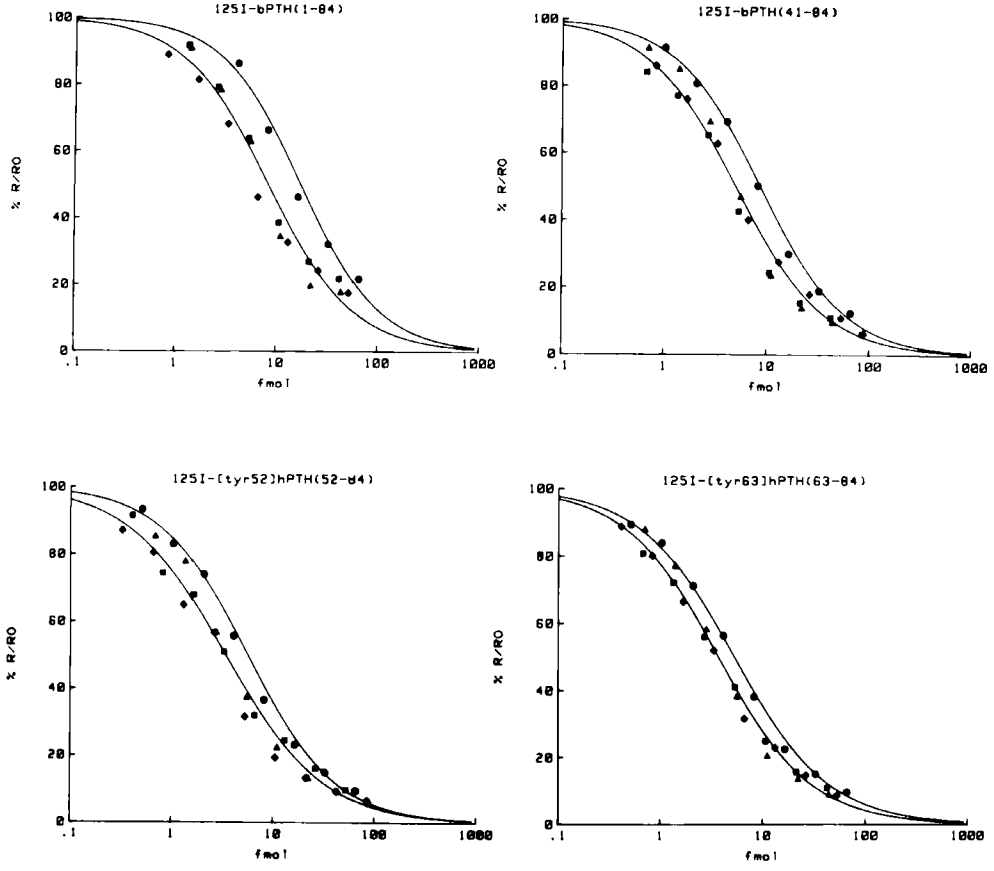

FIGURE 4 The characteristics of the 4 assays obtained by using 4 different tracers are illustrated. In each case, the displacement of the tracer obtained with hPTH(1-84) (●) is compared with the one obtained with fragments hPTH(41-84) (◆), hPTH(52-84) (▲) and hPTH(63-84) (■). The results are means of 2 or 3 different assays. Assay characteristics are analyzed in Table 1.

concentration of hPTH(1-84) or of fragments hPTH(...41-84), hPTH(53-84) or hPTH(64-84). hPTH(1-34) and hPTH(44-68) did not displace the various tracers. A detailed analysis of these displacement curves can be seen in Table I. Only the results obtained with hPTH(...41-84) are analyzed in Table I, since the displacement was the same with all fragments.

As can be seen, the titer of the antiserum giving nearly 30% binding of the tracer increased from 1/20,000 with ^{125}I-bPTH(1-84) to 1/50,000 with ^{125}I-{tyr^{52}}hPTH(52-84) or ^{125}I-{tyr^{63}}hPTH(63-84). The damage seen with each tracer decreased from 16.0±6.2% with ^{125}I-bPTH(1-84) to less than 3% with all other tracers. The sensitivity of each assay also improved with the smaller tracers. The amounts of hPTH(1-84) or hPTH(41-84) necessary to displace 50% of the tracer were 13.2±.8 and 6.3±1.0 fmol respectively with tracer ^{125}I-bPTH(1-84), while similar results could be achieved with 5.0±.4 or 3.5±1.2 fmol in the case of ^{125}I-{tyr^{63}}hPTH(63-84). In these various assays, fragments were always more potent than hPTH(1-84) on a molar basis in displacing the tracer, but the molar ratio of hPTH(1-84) to hPTH(41-84) improved with the smaller tracers, being 2.17±.43 for ^{125}I-bPTH(1-84) and 1.39±.08 for ^{125}I-{tyr^{63}}hPTH(63-84). This greater reactivity of the fragments with the larger tracers should result in higher values when sera are assayed with these tracers. This was verified with our two samples in the various assays (Table II). Significantly higher values are seen with ^{125}I-bPTH(1-84) as tracer, the lowest being with ^{125}I-{tyr^{52}}hPTH (52-84) and ^{125}I-{tyr^{63}}hPTH(63-84).

DISCUSSION

This study was undertaken to see how the molecular weight of various h or bPTH tracers, containing the 68-84 sequence of the b or

hPTH molecule, could affect the results of a hPTH radioimmunoassay specific for the 68-84 region of the hPTH structure.

The largest possible tracer, ^{125}I-bPTH(1-84), was obtained by iodinating natural bPTH(1-84). Two synthetic commercially available hPTH peptides, {tyr^{52}}hPTH(52-84) and {tyr^{63}}hPTH(63-84), were also iodinated. The other tracer was produced by incubating ^{125}I-bPTH(1-84) with plasma membranes of rat kidney cortex. Previous publications (22,23,24) have suggested that bovine (23,24) or rat (22) kidney membranes were able to generate large carboxylterminal fragment(s) from intact bPTH(1-84). With the conditions defined by others (23), we have been able to generate a large carboxylterminal fragment peak from ^{125}I-bPTH(1-84), as defined by gel chromatography. The major fragment present in this peak started at position 41 of the bPTH structure when studied by sequence analysis (20), and differed slightly from those obtained with bovine membranes under similar conditions (23). Large carboxylterminal fragment(s) were also produced from hPTH(1-84). Their elution position was similar to the one of iodinated fragment(s) generated from ^{125}I-bPTH(1-84), but their exact structure could not be studied. We assumed that the carboxylterminal end of b and hPTH fragments was probably intact since both peptides reacted fully in our 68-84 immunoassay. They were called ^{125}I-bPTH(41-84) and bPTH(...41-84).

We decided to use antiserum C-52 in these studies. This antiserum only reacts with antigenic sites on the carboxylterminal sequence of the b and hPTH structure and with none on the aminoterminal (9). Displacement of the ^{125}I-bPTH(1-84) tracer with hPTH(44-68), hPTH(53-84) and hPTH(64-84) have disclosed a first antigenic site in the sequence 44-53 and a second in the sequence 68-84. Regionspecificity

was achieved by saturating (6) the less sensitive mid-carboxylterminal component with an excess of synthetic hPTH(44-68). When treated in this way, this antiserum became specific for the 68-84 sequence of the b or hPTH structure, as shown with the various tracers studied.

The amount of charcoal-dextran necessary to achieve separation varied inversely with the molecular weight of each tracer. Furthermore, the damage, or amount of tracer bound in the absence of antiserum, remained quantitatively important for ^{125}I-bPTH(1-84) ($16.0\pm6.2\%$) but decreased with all the other tracers to less than 3.5%. It is interesting to note that ^{125}I-bPTH(41-84) was generated from ^{125}I-bPTH(1-84) and that cleavage of the aminoterminal sequence resulted in a 80% decrease in damage. This suggests that it is the aminoterminal sequence which is mainly involved with this phenomenon.

The four tracers selected were then studied in the 68-84 regionspecific radioimmunoassay. A first major finding was made: the titer of the antiserum necessary to achieve 30% specific binding of each tracer varied inversely with the molecular weight of the various tracers. A titer of 1/20,000 was necessary for ^{125}I-bPTH(1-84) but only one of 1/50,000 for ^{125}I-{tyr^{52}}hPTH(52-84) or ^{125}I-{tyr^{63}}hPTH (63-84) with an intermediary titer of 1/30,000 for ^{125}I-bPTH(41-84). The reason for this is not clear. A greater affinity of the antiserum for late carboxylterminal fragments might reflect the fact that this antiserum was developed against those fragments, even if partially purified bPTH(1-84) was used for immunization. This finding is important, since practically speaking, it means that with the low molecular weight tracers we can now perform 100,000 assay tubes/ml of antiserum where we could only perform 40,000 before with ^{125}I-bPTH(1-84).

The next finding was that those assays performed with the smaller tracers were more sensitive. One required 3.5 times less hPTH (1-84) or 2.29 times less hPTH(41-84) to achieve similar displacement of the ^{125}I-{tyr^{52}}hPTH(52-84) or ^{125}I-{tyr^{63}}hPTH(63-84) tracers when compared to tracer ^{125}I-bPTH(1-84). This increased sensitivity may be related to the higher antiserum titer achieved with smaller tracers.

The molar reactivity of the carboxylterminal fragment hPTH(...41-84) was always greater than that achieved by hPTH(1-84) with all the tracers. This difference in molar reactivity again improved with the smaller tracers, the molar ratio for equal displacement being 2.17 when ^{125}I-bPTH(1-84) was used as tracer and only 1.4 when ^{125}I-{tyr^{52}}hPTH (52-84) or ^{125}I-{tyr^{63}}hPTH(63-84) were used. One study has previously outlined a cleavage-associated enhancement of an antigenic site in the biologically active NH_2-terminal region of parathyroid hormone (25), and this may be pertinent to our findings with hPTH(41-84) and hPTH(1-84).

If the previous findings are exact, one would expect that the values obtained in a clinical assay with ^{125}I-bPTH(1-84) as the tracer and hPTH(1-84) as the standard would be higher than those obtained with ^{125}I-{tyr^{52}}hPTH(52-84) or ^{125}I-{tyr^{63}}hPTH(63-84). This was indeed the case with the 2 samples that were measured in the various assays.

In summary, our findings indicate that there are advantages to using small synthetic peptides as tracers in a radioimmunoassay specific to the 68-84 region of the hPTH structure. Among these, an increase in antiserum titer can be noted, together with a decrease in assay damage and an improvement in assay sensitivity and comparative molar reactivity of the various circulating forms of hPTH.

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