Studies of Circulating Parathyroid Hormone in Man by a Homologous Amino-terminal Specific Radioimmunoassay

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A homologous radioimmunoassay specific for amino-terminal portion of human parathyroid hormone(PTH) was developed in order to study the clearance of biologically active species of the hormone in the circulation in man. Characterization of the assay system with synthetic analogues of human PTH (1-34) indicated that the carboxyl-terminal region of human PTH(1-34) is an important recognition site. Plasma amino-terminal PTH levels were less than 0.3 ng/ml in all of 35 normal subjects. The levels were elevated above 0.3 ng/ml in 15 of 24 patients with primary hyperparathyroidism. In 5 patients in whom the levels were determined before and after parathyroidectomy, the elevated levels were all normalized within 60 minutes after the removal of adenomata. The disappearance of exogenous human PTH(1-34) was studied after intravenous administration in 17 patients with hypoparathyroidism. A graphical analysis of the data disclosed two major components of the disappearance curve with estimated half-disappearance time of 3 and 28 minutes respectively, suggesting that multiple mechanisms are involved in the clearance of the peptide from the circulation. These results demonstrate usefulness of homologous radioimmunoassay for human PTH(1-34) in diagnosis and management of hyperparathyroidism, as well as in studying the clearance of amino-terminal portion of PTH which is known to represent biological activity.

Key Words: Amino-terminal(1-34) fragment, Exogenous synthetic human PTH(1-34), Half-disappearance time

Circulating parathyroid hormone(PTH) is a mixture of intact hormone consisting of 84 amino-acids, carboxyl-terminal fragments and possibly amino-terminal fragments. Although the full biological activity exists in 34 amino-terminal residues, the predominant species of immunoactive PTH in the peripheral blood are carboxyl-terminal fragments that have longest half-lives. Most of radioimmunoassay for human PTH have been developed depending on the cross-reactivity of human PTH to antisera raised against bovine or porcine PTH. Problems existing in those assays have not only that they are heterologous in nature but also that they are usually specific for carboxyl-terminal portion of the molecule and major determinants of values in such assays are carboxyl-terminal fragments which are biologically inactive.

Little is known the origin, the nature and fate of amino-terminal fragments of PTH. There is disagreement whether amino-terminal fragment survives to be detected in the peripheral circulation. Studies of the clearance of exogenously administered synthetic amino-terminal fragment, mostly based on heterologous experimental system in quite limited numbers of animals or patients,

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have yielded somewhat conflicting results in estimation of half-disappearance time.\textsuperscript{1,6,7}

In attempts to investigate the clearance of biologically active species of PTH in man, we have developed homologous radioimmunoassay which is specific for amino-terminal portion of human PTH. In the present paper we describe the characteristics and clinical significance of the assay as well as the results from the studies of the clearance of exogenous amino-terminal fragment, synthetic human PTH(1-34), which was administered to patients with hypoparathyroidism.

SUBJECTS

28 patients with hypoparathyroidism (26 with idiopathic and 2 with postsurgical hypoparathyroidism), 7 patients with pseudohypoparathyroidism, 24 patients with primary hyperparathyroidism and 35 normal subjects were studied. Most of the patients with hypoparathyroidism had been treated with calcium lactate and/or vitamin D and were normocalcemic or slightly hypocalcemic. In 5 patients with primary hyperparathyroidism, serum amino-terminal PTH immunoreactivity was measured before and after parathyroidectomy.

MATERIALS AND METHODS

1) Synthetic human PTH(1-34) and analogues

Human PTH(1-34) (hPTH(1-34)) with the sequence proposed by Niall et al.\textsuperscript{8} as well as its synthetic analogues, Nle\textsuperscript{8},Nle\textsuperscript{18}-hPTH(1-34), Tyr\textsuperscript{-1},Nle\textsuperscript{8},Nle\textsuperscript{18}-hPTH(1-34) and Nle\textsuperscript{8},Nle\textsuperscript{18},Tyr\textsuperscript{34}-hPTH(1-34) were prepared by liquid phase synthesis at Toyo Jozo Institute (Shizuoka). HPTH(53-84) was purchased from Peptide Research Institute (Minoo, Osaka).

2) Radioimmunoassay

The antisera were raised by immunizing guinea pigs with the synthetic hPTH(1-34) emulsified in complete Freund's adjuvant. The final dilution of the antisera used in the present studies was 1:15,000. Radioiodination of synthetic hPTH(1-34) was performed by a modification of chro-lamin T method.\textsuperscript{9} Antisera and \textsuperscript{125}I-hPTH(1-34) were diluted with assay buffer: 0.05M sodium phosphate, pH 7.5 containing 0.01 M EDTA-Na\textsubscript{2} and 1% bovine serum albumin. The standard synthetic hPTH(1-34) was diluted in pooled plasma from patients with idiopathic hypoparathyroidism. In regular assays 100 \mu l of plasma samples or standards, 100 \mu l of antisera and 200 \mu l of assay buffer were incubated at 4\textdegree C for 2 days. After 100 \mu l (100,000 cpm) of \textsuperscript{125}I-hPTH(1-34) was added, incubation continued for another 2 days. Separation of bound and free tracer was accomplished by double antibody method. In studies to characterize the assay system, standard hPTH(1-34) was simply replaced by synthetic analogues of hPTH(1-34).

3) Disappearance of exogenous human PTH(1-34) from circulation

In 17 patients with hypoparathyroidism, 20 \mu g of synthetic human PTH(1-34) in 3 ml of saline was administered intravenously as a single bolus injection over 1 minute at 10:00 a.m. after overnight fast. Blood samples were drawn 30 minutes before and 5, 10, 15, 30 and 60 minutes after administration and immediately transferred into glass tubes containing 10 \mu moles of EDTA-Na\textsubscript{2}. Plasma was separated and stored at -20\textdegree C until analysis.

RESULTS

1) Characterization of radioimmunoassay

A typical standard curve is shown in Fig. 1. The detection limit for hPTH(1-34) was 0.1 ng/ml. The assay was further characterized with synthetic analogues of human PTH(1-34). As shown in the same figure, Nle\textsuperscript{8},Nle\textsuperscript{18}-hPTH(1-34) and Tyr\textsuperscript{-1},Nle\textsuperscript{8},Nle\textsuperscript{18}-hPTH(1-34) disclosed the tracer in almost identical fashion as hPTH(1-34). Nle\textsuperscript{8},Nle\textsuperscript{18},Tyr\textsuperscript{34}-hPTH(1-34), however, was much less potent in displacing the tracer. Amino-terminal specificity of this assay was confirmed by lack of inhibitory effect of hPTH(1-34) on binding of the tracer.

2) Plasma amino-terminal PTH immunoreactivity

In 35 normal subjects plasma concentrations of amino-terminal PTH immunoreactivity ranged from undetectable (i.e. < 0.1 ng/ml) to 0.3 ng/ml, being undetectable in 24 subjects (Fig. 2). In 28 patients with idiopathic or postsurgical hypoparathyroidism amino-terminal PTH immunoreactivity

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Fig 1. Reactivity of synthetic human PTH analogues in the amino-terminal specific radio-immunoassay: hPTH(1-34) (●), Nle⁶,Nle¹⁸-hPTH(1-34) (▲), Tyr¹,Nle⁶,Nle¹⁸-hPTH(1-34) (●), Nle⁶,Nle¹⁸,Tyr⁴⁰-hPTH(1-34) (■), hPTH(53-84) (○).

Fig 2. Plasma amino-terminal PTH levels in normal subjects and patients with parathyroid diseases. Dotted line indicates the limit of detection.

was undetectable except 5 patients, in whom concentrations were below 0.2 ng/ml. In 4 of 7 patients with pseudohypoparathyroidism and 15 of 24 patients with primary hyperparathyroidism amino-terminal PTH immunoreactivity were

Fig 3. Plasma amino-terminal PTH levels before and 60 min after parathyroidectomy in patients with primary hyperparathyroidism. Disrupted line indicates the highest level in the normal subject.
Fig. 4. Plasma amino-terminal PTH levels after parathyroidectomy in a patient with primary hyperparathyroidism. Dotted line indicates the limit of detection.

Elevated above the upper limit in normal subjects. When plasma amino-terminal PTH immunoreactivity were determined pre- and postoperatively in 5 patients with primary hyperparathyroidism, elevated levels were normalized in all patients within 60 minutes after removal of parathyroid adenomata (Fig. 3). A time course in one patient of these is depicted in Fig. 4. The amino-terminal PTH immunoreactivity decreased by 20% of the preoperative level at 6 minutes and become undetectable at 15 minutes after removal of single adenoma. A half-disappearance time was roughly estimated to be less than 9 minutes.

3) Disappearance of exogenous hPTH(1-34) from circulation

In 17 patients with idiopathic or postsurgical hypoparathyroidism 20 μg of synthetic hPTH(1-34) was injected intravenously and plasma concentrations of amino-terminal immunoreactivity were monitored. The preinjection levels were below 0.1 ng/ml in all these patients. The concentrations of amino-terminal PTH at 5 minutes after injection ranged from 1.25 to 5.1 ng/ml (mean

Fig 5. Disappearance of amino-terminal PTH from the circulation of patients with hypoparathyroidism after intravenous injection of 20 μg of synthetic human PTH(1-34). Time 0 indicates the end of injection. Results are shown as mean ± SEM for 17 patients.

Fig 6. Graphical analysis of the data shown in Fig 5. The curve is composed of two components indicated by dotted lines.
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± SEM, 2.63 ± 0.26 ng/ml) and thereafter fell in a multiexponential manner as shown in Fig. 5. At 15 minutes after injection the concentrations were 45.2% (in average) of those at 5 minutes and at 60 minutes the concentrations almost returned to preinjection levels.

When these data were analysed on a semilogarithmic plot, there were two major components, one with a half-disappearance time (T\(\frac{1}{2}\)) of 3 minutes and another with T\(\frac{1}{2}\) of 28 minutes (Fig. 6).

DISCUSSION

In our homologous radioimmunoassay for human PTH(1-34), Nle\(^8\),Nle\(^{18}\)-hPTH(1-34) and Try\(^{-1}\),Nle\(^8\),Nle\(^{18}\)-hPTH(1-34) were almost as potent as native hPTH(1-34) in displacing \(^{125}\)I-hPTH(1-34). Nle\(^{8}\),Nle\(^{18}\),Tyr\(^{34}\)-hPTH(1-34), on the other hand, reacted only incompletely indicating that the carboxyl-terminal structure of hTPH (1-34) is an important recognition site.

Although immunoreactive PTH(1-34) levels in patients with primary hyperparathyroidism were partially overlapped with those in normal subjects, usefulness of this assay is not impaired because this peptide is revealed to be biological active one.

Segre et al. reported that bovine PTH injected to dogs was degradated rapidly into fragments and sites of cleavage were within sequence 33-37.\(^{10}\) No evidence has yet been available to answer whether the same type of cleavage occurs to endogenous human PTH, but Canterbury et al. found, in gel-chromatography of human plasma, a peak of immunoreactivity having an estimated molecular size of 4,500, which was proved to activate rat renal cortical adenyl cyclase.\(^{9}\) Based on these informations, it could be considered that our assay recognize amino-terminal fragment as demonstrated by Canterbury et al., as well as intact hormone, but underestimate smaller fragments lacking the carboxyl-terminal structure of PTH(1-34), if they exist in the circulation.

The elevated amino-terminal PTH immunoreactivity in patients with primary hyperparathyroidism all fell into normal range within 60 minutes after removal of adenomata. In one of the patients, T\(\frac{1}{2}\) for endogenous amino-terminal PTH immunoreactivity was roughly estimated to be less than 9 minutes. Papapoulos et al. made similar studies in two patients with primary hyperparathyroidism and reported that T\(\frac{1}{2}\) for endogenous amino-terminal PTH was 2.5 and 3.0 minutes respectively.\(^{9}\) More detail studies are required, however, since amino-terminal PTH immunoreactivity in circulation is supposed to consist of at least two molecular forms that may have different half-lives.

Silverman and Yalow reported that T\(\frac{1}{2}\) of exogenous synthetic bovine PTH(1-34) in a dog was about 5 minutes during earliest period after injection.\(^{11}\) Papapoulos et al. administered synthetic hPTH(1-34) in two normal subjects and determined T\(\frac{1}{2}\) to be 2.5 minutes.\(^{9}\) The disappearance curve of the peptide in one of their subjects, however, was also multiexponential as seen in our patients and T\(\frac{1}{2}\) was calculated from data in the initial phase. Hruska et al. observed multiexponential decline of both bovine PTH(1-34) and bovine PTH(1-84) after administration to dogs.\(^{6}\) They reported T\(\frac{1}{2}\) for PTH (1-34) and PTH(1-84) to be 4 and 3 minutes in the initial phase and 16 and 94 minutes in the late phase respectively. Although T\(\frac{1}{2}\) for two peptides in the late phase were quite different from each other and were markedly prolonged by induced renal failure, T\(\frac{1}{2}\) in the initial phase were essentially the same for both peptides and were not influenced by renal failure. These findings, taken together, may allow an interpretation that initial rapid disappearance of PTH(1-34) after administration represents diffusion into distribution space and slower disappearance observed in Hruska’s and our studies reflects uptake of the peptide by tissues such as kidney and bone.\(^{11,12}\)

The interpretation of discrepancy in half-disappearance time for endogenous and exogenous PTH(1-34) is considered as following. Firstly, the two immunoreactive PTH(1-34) are not necessarily the same peptide because endogenous immunoreactive PTH(1-34) is a mixture of various fragments. Secondly, to be more important, in primary hyperparathyroidism tissues such as kidney and bone are already saturated by bio-
logically active PTH(1-34), so, when the adenoma are removed most of immunoreactive PTH (1-34) is rapidly disappeared from circulation. On the other hand, in patients with hypoparathyroidism who have no immunoreactive PTH (1-34) exogenous synthetic PTH(1-34) diffuses to distribution space in initial rapid phase and then is taken by tissues in slower phase.

Our results that exogenous hPTH(1-34) has $T_{1/2}$ longer than 20 minutes and can be detected even at 60 minutes after administration in a large dose are not surprising, since endogenous amino-terminal fragment has been found in circulation of patients with hyperparathyroidism.$^{1,2}$ Experiments in animals in vivo and in vitro have shown amino-terminal fragment is not only generated in the kidney$^6$ and the liver$^{13,14}$ but also secreted directly from parathyroid gland.$^{15}$ If the amino-terminal fragment survives in circulation even in a small quantity, it may serve a considerable portion of total PTH bioactivity.

The availability of hPTH(1-34) and variety of its synthetic analogues will enable better understanding of the clearance of PTH in man.

REFERENCES