

Review Article

PTH Assays: Understanding What We Have and Forecasting What We Will Have

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Parathyroid hormone (PTH) assays have evolved continuously for the last 50 years. Since the first radioimmunoassay was described in 1963, several assays based on immunological identification have been published (first generation assays). The routine assays used nowadays are immunometric “sandwich-type”. They are based on two different monoclonal antibodies, one amino-terminal and the other carboxyl terminal specific. These second generation assays are widely available and adapted to most of the automation platforms. The specificity of the amino terminal antibody defines if the immunometric assay measures only the bioactive PTH circulating form (including the first amino terminal amino acids) or the “intact” PTH, which includes, besides bioactive PTH, other “long” carboxyl-terminal forms, for example, 7–84-PTH. Assays for “intact” PTH are the most commonly available and the potential advantage of the bioactive PTH assays is still debatable. Next generation of assays will be based on different principles, mainly mass spectrometry in samples submitted to a prior purification and fragmentation steps. These assays will provide information about the whole spectra of PTH peptides in circulation, with a significant increase of the information regarding this biologically important peptide hormone.

1. Introduction

Parathyroid hormone (PTH) is a linear peptide consisting of 84 amino acids and produced by the parathyroid cells. It plays a critical role in the calcium metabolism, and its receptor (PTH1R) is present in several tissues, with special importance in renal tubules and bone cells. The classical biological activity of PTH, mediated by PTHR1 activation, is dependent on the presence of an intact amino terminal sequence, mainly the first amino acids. Yet PTH present in circulation is very heterogeneous, and this heterogeneity is the consequence of a complex metabolism that starts in the parathyroid cells and continues in other tissues, mainly in the kidneys and liver [1, 2]. The result of this complex metabolism is the presence of a circulation pool of “PTH peptides,” not only in pathological conditions, but also in normal individuals. This phenomenon is particularly important in patients with end-stage renal disease, where PTH fragments, with marked predominance of the carboxyl terminal ones, are present in great quantities in comparison to the intact 1–84 form (Figure 1) [3]. One important point is the fact

that the biologically active forms have very short half-lives, compared to the carboxyl terminal fragments (devoid of the amino terminal amino acids) that have longer half-lives and accumulate in circulation as glomerular filtration declines.

The recent demonstration of functional carboxyl-terminal receptors for PTH, and of their potential physiological importance, brought a new challenge in the interpretation of PTH results [4–6]. The correct definition of the form recognized by the assay employed for serum PTH measurement becomes very important.

PTH measurement has evolved alongside immunoassay methods for almost 50 years, since the first practical method was published in 1963 [7]. It is a long story, and the accumulated knowledge about physiology and metabolism of the molecule provided new tools for the development of new assays in a continuous feedback process.

2. Evolution of PTH Assays

We can divide, for the sake of simplicity, the evolution of PTH assays in three groups of methods: the competitive

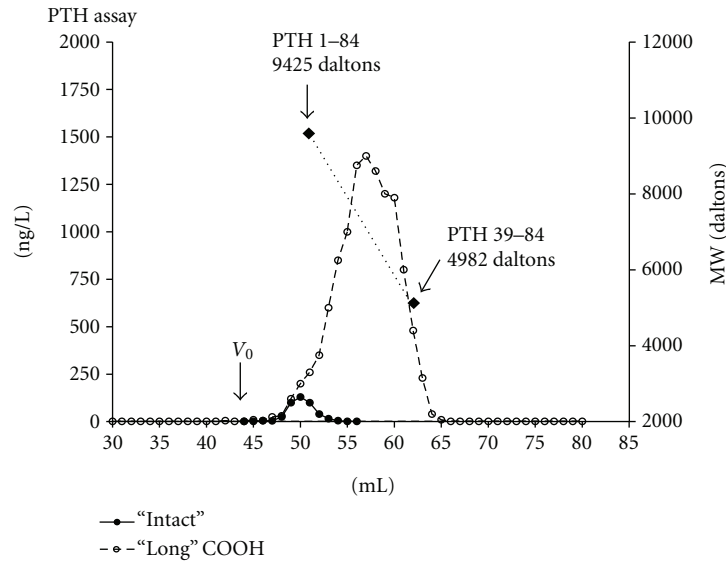


FIGURE 1: Elution profile of a serum sample from a patient with end-stage renal disease loaded on a Hiload Superdex 30 1.6×60 column. PTH was measured using a carboxyl terminal assay (“long” COOH) and a routine “intact” PTH assay.

immunoassays (1st generation), the immunometric assays (2nd generation), and the new assays recently described using mass spectrometry (3rd generation). In the first case a single polyclonal antibody competes for labeled PTH and the serum forms. In the 2nd-generation assays, two distinct antibodies (usually monoclonal), directed against different epitopes, bind the PTH forms present in the sample. One of the antibodies is bound to a solid phase, and the other is labeled. In the third type of assays, the recognition is based on other principles (mass spectrometry) in serum samples previously purified.

The first description of a radioimmunoassay for PTH, by Berson et al. in 1963 [7] was a tremendous breakthrough and initiated a revolution in the diagnosis of parathyroid conditions. Several other radioimmunoassays were described in sequence, all based on antibodies raised against PTH extracted from bovine or porcine parathyroid glands. These heterogeneous 1st-generation assays had important limitations, including the difficulty of producing antibodies with sufficient affinity and defined specificity. These difficulties derived from the fact that bioactive PTH circulates in concentrations that in normal individuals are below 10 pmol/L, which is *per se* a methodology challenge. The specificity of these first antibodies was directed mainly against carboxyl fragments, the PTH form predominant in the gland extracts used for immunization, for standard preparation and for labeling. Nevertheless, immunoassays with reasonable characteristics and clinical correlation were described [8, 9]. These carboxyl terminal assays had a major limitation that was related to the retention of these fragments with decrease in glomerular filtration, rendering their use in chronic renal disease patients almost invalid [10, 11].

With the description, by Potts et al. [12], that a synthetic peptide comprising the 1–34 sequence of PTH was biologically active and equipotent to the complete 1–84 peptide, an effort to develop amino-terminal-specific assays ensued. The

first such assay was published by Desplan et al. in 1977 [13], followed by other publications [14, 15]. We had an extensive experience with the development and use of an amino-terminal-specific radioimmunoassay for PTH. The assay was based on egg-yolk-extracted antibodies, and despite problems in sensitivity, the assay was diagnostically superior to the most available carboxyl terminal radioimmunoassay [16–18].

3. Immunometric Assays: A New Benchmark

The description of the first immunometric assay for PTH (noncompetitive, “sandwich” type assay) by Nussbaum et al. in 1987 [19] was a major breakthrough, and the sensitivity and specificity problems were considered overcome. Immunometric assays (2nd generation) are based on the recognition of PTH by two different antibodies, one carboxyl terminal and the other amino terminal specific. Initially these assays were considered to recognize only intact PTH (1–84) and considered to be the gold standard for the definition of parathyroid dysfunctions [20]. Nevertheless, publications by the group of D’Amour et al. showed that it could not be exactly the case [21, 22]. These authors showed that PTH forms with deletion of the first amino terminal amino acids (mainly 7–84) are present in significant concentration, particular in patients with renal insufficiency. We confirmed these observations using an in-house immunofluorometric assay and HPLC separation for the PTH forms in circulation [23]. Since the biological activity of PTH, via PTHR1 binding, depends on the presence of these first amino acids, these 7–84 forms have no classical PTH biological activity. The reason why the “intact” assays measured also these deleted forms relates to the specificity of the amino terminal antibody used, since most of them are directed to an antigenic site located around amino acids 20 to 25 (amino terminal antibody type 1 in Figure 2). This region of the molecule is more

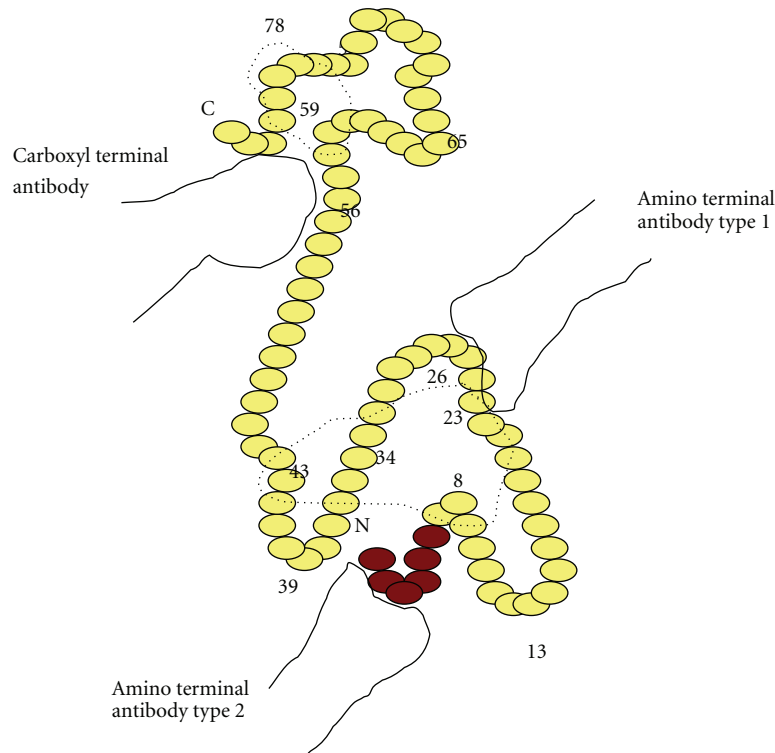


FIGURE 2: Schematic presentation of the regions of the PTH molecule recognized by the antibodies used in the immunometric assay design. Amino terminal antibody type 1 represents the most common antibodies employed for amino terminal sequence recognition. Amino terminal antibody type 2 refers to antibodies directed to the first amino acids of PTH molecule.

antigenic, and a successful immunization (mice or other animal) usually produces antibodies with that specificity. In order to have an assay recognizing only the biologically active 1–84 PTH form, the amino terminal antibody used should recognize the first amino acids (amino terminal antibody type 2 in Figure 2). Assays with this specificity were described, are available commercially, and were extensively studied against the standard immunometric assays [24, 25]. The conclusion of all these studies was that besides having a lower reference range, these “biologically active” 1–84 PTH assays showed no clear advantage over the more available, less expensive, and extensively validated “intact” assays [26].

4. Current State

Immunometric assays for measurement of “intact” serum PTH are widely available in clinical laboratories, have good sensitivity and reproducibility, and well-defined normal reference values [27, 28]. One point is still debatable and relates to the use of these assays in patients with hyperparathyroidism secondary to renal insufficiency, since as described by Quarles et al. in 1994, dissociation between serum PTH levels and bone abnormality exists in these patients [29]. One logical solution, the use of the immunometric assays that measures only the “biologically active” 1–84 PTH form, showed no diagnostic advantage [30]. The conclusion is that some form of skeletal resistance exists in this condition, and it may be in part explained by the

interaction of PTH molecules that bind to PTHR1 but do not activate it. These somewhat unexpected observations may be related to the notion that PTH should be viewed as a polyhormone, a concept first raised by Mallette in 1991 [31]. The wide range of circulating forms of PTH may include forms with biological activity different from those expected by the activation of PTHR1 [3–6]. This observation can be further stressed by the recent description, in some parathyroid carcinoma patients, of circulating forms of PTH that are able to activate PTHR1 but are not recognized by the current “intact” assays [32].

5. Future Prospects

The additional information potentially provided by the measurement of the various forms of circulating PTH peptides is very attractive, but attaining this goal using immunometric assays would require several different assays. The use of liquid chromatography coupled to tandem mass spectrometry in the clinical laboratory is rapidly maturing and is already in routine in diagnostic laboratories for steroid hormones [33]. The enormous potential of these techniques already led to the development of methods that associate immunoaffinity, *in situ* digestion, and mass spectrometry for the discrimination and quantification of the pool of circulating PTH forms [34, 35]. We foresee these techniques (3rd generation) as the next step in PTH quantification, with broad new clinical applications.

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