Available online at www.sciencedirect.com

SCIENCE DIRECT®

Clinica Chimica Acta 366 (2006) 81 – 89

www.elsevier.com/locate/clinchim

Review

Practical considerations in PTH testing

Jean-Claude Souberbielle [a,*], Gérard Friedlander [a], Catherine Cormier [b]

[a] *Laboratoire d'Explorations Fonctionnelles, hôpital Necker-Enfants Malades, AP-HP, 149 rue de Sèvres, 75015 Paris, France*
[b] *Service de rhumatologie, hôpital Cochin, rue du faubourg St-Jacques, AP-HP, 75014 Paris, France*

Received 24 June 2005; received in revised form 18 October 2005; accepted 20 October 2005
Available online 28 November 2005

Abstract

New knowledge concerning PTH biology have accumulated during the past few years. The finding that the so-called "intact" PTH assays measure a "non-1–84" PTH fragment in addition to full-length PTH has led to the development of new assays. These new assays, which were initially thought to measure 1–84 PTH only, have been shown to recognize also another PTH species called "amino-PTH". As the various names given to the different assay methods are highly confusing, there is a need for a simplified nomenclature. A simple way would be to identify the older "intact" PTH assays as second-generation assays and the new assays (Whole, CAP, BioIntact) as third-generation assays. Although of considerable potential interest for the comprehension of PTH physiology, the third-generation PTH assays have not yet proved to be superior to the second-generation assays in clinical practice. There is thus currently no recommendation to switch from the second-generation to the third-generation assays in clinical practice, or to use a ratio derived from the concommitent measurement of PTH with both assay-generation. Because second- and third-generation PTH assays are usually highly correlated, significant differences in the clinical information provided by these methods are unlikely. However, our opinion is that more definitive bone biopsy studies in dialyzed patients selected according to their bone- and calcium-related treatment are still needed to reach a consensus. Finally, we have proposed that PTH reference values should be established in healthy subjects with a normal vitamin D status. This supposes that 25OHD is measured in the reference population beforehand, and that the subjects with vitamin D insufficiency are eliminated from the reference group. Although more complicated than the usual way to establish normative data, we have shown that it decreases the upper limit of normal by 25–35%, enhancing thus the diagnostic sensitivity for hyperparathyroidism without a decrease in specificity.

Keywords: Parathyroid hormone; Immunoassay; Vitamin D; Renal osteodystrophy; Hyperparathyroidism

Contents

* Corresponding author. Tel.: +33 1 44 38 17 43.
E-mail address: jean-claude.souberbielle@nck.ap-hop-paris.fr (J.-C. Souberbielle).

0009-8981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.cca.2005.10.010

1. Introduction

Parathyroid hormone (PTH) is a single-chain 84 amino-acid peptide hormone encoded by a gene on the short arm of chromosome 11 and produced by the parathyroid glands in response to a decrease in the extracellular concentration of ionized calcium (Ca++). Its main role is to increase serum Ca++, which is achieved by stimulating the release of calcium from bone and its renal reabsorption in the distal tubule. PTH also stimulates the activity of the 1-alpha hydroxylase enzyme in the renal proximal tubule, enhancing the synthesis of 1.25 dihydroxy-vitamin D (1.25OH2D), the active metabolite of vitamin D, which in turn increases intestinal absorption of calcium and exerts an endocrine feed-back on the secretion of PTH at the parathyroid level. PTH also decreases the renal reabsorption of phosphate in the proximal tubule, thereby decreasing serum phosphate. Furthermore, PTH stimulates bone formation, and this property is now used in clinical practice for the treatment of osteoporosis [1]. PTH exerts these actions through a G-protein coupled receptor, the PTH/PTHrP receptor (or PTHR1) [2]. It has been demonstrated that the very first N-terminal amino-acids of the PTH molecule are indispensable for this interaction [3]. Besides full-length 1–84 PTH, various PTH fragments are present in blood, whose exact composition and possible function are not yet fully elucidated. PTH measurement is routinely prescribed in patients with chronic renal failure (CRF) to identify renal osteodystrophy (ROD) subtypes and adapt treatment, and in non-renal patients to explore any disorder of calcium–phosphate metabolism. Although not yet consensual, it was recently proposed to measure serum PTH in any postmenopausal osteoporotic women, even if normocalcemic, to exclude a possible treatable cause of secondary osteoporosis [4]. During the past 5–6 years, new PTH assays became available and new concepts concerning PTH reference values have emerged. The aim of the present review is to discuss these two points and their possible implication in the interpretation of PTH concentrations.

2. The different PTH assays and what they measure

First-generation PTH assays were radio-immunoassays (RIA) [5] using polyclonal antibodies directed mainly, but not exclusively, against synthetic C-terminal (such as 53–84 PTH) or Mid-region (such as 44–68 PTH) PTH fragments. These fragments, which are mainly produced in the liver by the catabolism in the Kupffer cells and were thought to be inactive, are also secreted by the parathyroids [6]. They are eliminated by the kidney, have a very longer half-life than 1–84 PTH and accumulate in CRF patients [7]. The consequence is that, in CRF patients and specially in those undergoing dialysis, PTH concentrations measured with these first-generation assays were always greatly increased, even in those patients clearly identified as having low turnover bone disease, a condition associated with a defect in PTH action. Furthermore, these assays had a poor analytical sensitivity in the low concentrations rendering discrimination between low- and normal-levels difficult. For these reasons, and although they are still highly useful to understand the physiology of the C-terminal fragments, the first-generation PTH assays are currently considered obsolete for the clinical practice and are seldom used. During the mid-1980's, the first second-generation PTH assay, the Allegro assay, became available [8]. This immunoradiometric assay (IRMA) uses two different antibodies. The capture antibody coated to a plastic bead is directed against the 39–84 portion of the PTH molecule, whereas the 125-I labelled antibody recognizes mainly the 13–24 portion of the PTH molecule [9]. This assay is thus unable to measure the C-terminal or mid-fragments (such as 53–84 or 44–68) which were measured with the first-generation assays [8]. During the following years, several similar assays, either IRMA or "non-radioactive" immunometric assays, became available [10–12], some of them on fully automated immunoanalyzers [13,14]. Some of these assays use an anti-N-terminal antibody directed, like in the Allegro assay, towards the proximal 13–24 portion of the hormone, whereas others, like the Elecsys intact PTH assay, recognize a more distal epitope in the 26–32 portion [9]. These second-generation assays were globally called "intact" PTH assays as they were thought to measure only the full-length 1–84 PTH. Although producing far more clinically satisfying data than first-generation assays, the second-generation assays were rapidly shown to present some limitations. In particular, several reports suggested that they overestimated the degree of secondary hyperparathyroidism in CRF patients [15,16]. Indeed, it was not understood why a haemodialized patient with histological features of low turnover bone disease may have an "intact" PTH concentration as high as 400–500 pg/mL. One possible explanation involving PTH assays came from the demonstration that several "intact" PTH assays recognized with various cross-reactivities (from approximately 50% to 100%) a PTH molecule, different from 1–84 PTH, which co-eluted in HPLC with a synthetic 7–84 PTH fragment [17]. In the recent literature, this fragment is identified either as "non-1–84" PTH, "N-terminal truncated" PTH or "7–84" PTH. Throughout the present review, we identify this fragment as "non-1–84 PTH". Very recently published data indicate that "non-1–84 PTH" is composed of a family of fragments. The longest and the shortest fragment starts at position 4 and at position 15, respectively, whereas the major component is a peptide starting at position 7 [18]. In 1999, the first third-generation PTH assay was developed by Scantibodies Laboratories [19]. This IRMA, called Whole PTH assay, uses an anti-C-terminal antibody similar to those of the "intact" PTH assays, but an anti-N-terminal antibody directed against the very first amino-acids (1 to 4), and does not measure the "non-1–84" PTH fragment [19,20]. It was shown to produce lower serum concentrations than the