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Review

Update on parathyroid hormone: New tests and new challenges for external quality assessment

David E.C. Cole [a,b,c,d], Sharon Webb [e], Pak-Cheung Chan [a,b,*]

[a] Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada
[b] Department of Clinical Pathology, Sunnybrook Health Sciences Center, Toronto, ON, Canada
[c] Department of Medicine, Ontario Medical Association, Toronto, ON, Canada
[d] Department of Pediatrics, University of Toronto, Ontario Medical Association, Toronto, ON, Canada
[e] Quality Management Program–Laboratory Services, Ontario Medical Association, Toronto, ON, Canada

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Abstract

It is now 43 years since Berson and Yalow published the first radio-immunoassay (RIA) for parathyroid hormone (PTH) [S.A. Berson, R.S. Yalow, G.D. Aurbach, J.T. Potts, Immunoassay of bovine and human parathyroid hormone. *Proc Natl Acad Sci U S A* 49 (1963) 613–617] [1]. Since then, there have been marked advances in our understanding of this peptide hormone, its mechanism of action and biological regulation [J.T. Potts, Parathyroid hormone: past and present. *J. Endocrinol*. 187 (2005) 311–325] [2]. PTH has become a routine assay in tertiary care hospitals and is an essential element in the management of chronic kidney disease, parathyroid disorders and the investigation of abnormalities in calcium homeostasis. Despite continuing technological advances in PTH measurement, analyte heterogeneity remains a problem, while improved turnaround time and better precision are constantly escalating clinical demands. This mini-review begins with a brief update of current knowledge on PTH, followed by a summary of a recent Ontario-wide External Quality Assurance (EQA) survey, and concludes with comments on utilization trends, current and future.

Keywords: Parathyroid hormone (PTH); Calcium homeostasis; Utilization

Contents

Introduction

Parathyroid hormone (PTH) is a polypeptide hormone that plays a central role in the maintenance of calcium and phosphate homeostasis and bone health. It acts to maintain bone and mineral homeostasis largely through four mechanisms: (i) elevation of blood calcium by increasing osteoclastic bone resorption, (ii) enhancement of renal calcium reabsorption, (iii) stimulation of renal 1,25-dihydroxyvitamin D synthesis, leading to increased intestinal calcium absorption, and (iv) promotion of phosphaturia via inhibition of renal tubular transepithelial phosphate reabsorption [3]. These biologic responses are down-

* Corresponding author. Rm B204, Sunnybrook Health Sciences Center, 2075 Bayview Avenue, Toronto ON, Canada M4N 3M5. Fax: +1 416 4806120.
E-mail address: pc.chan@sunnybrook.ca (P.-C. Chan).

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stream actions following the binding of the NH_2-terminal portion of the mature PTH molecule or fragments to a common PTH/PTH related protein (PTHrP) receptor, PTH1R, on the plasma membranes of target tissue cells [3–5]. Signals of PTH1R activation following ligand binding may be transduced via either the classic pathway involving G-protein (Gαq/11), adenylyl cyclase and phospholipase C or the non-classic pathway involving G-protein (GαS), Erk, phospholipase A_2 and phospholipase D under the influence of a Na^+/H^+ exchange regulatory factor (NHERF1) in a cell specific manner [34]. While the first two amino acid residues of PTH are required for PTH1R signaling through adenylyl cyclase, evidence now exists for distinct carboxyl- or C-terminal PTH receptors, which confer antagonistic or hypocalcemic effects that oppose some of the actions of intact PTH or NH_2-terminal fragments, with potential clinical and pharmacological implications [3,6]. While other types of N-terminal PTH receptors (non-PTH1R) and non-classical actions of PTH have been described, they are beyond the scope of this mini-review and will not be discussed here. Interested readers may refer to other recent publications [3,36].

Biology of PTH

Hypocalcemia stimulates PTH secretion into the circulation through intracellular signals from the calcium-sensing receptor embedded in the plasma membrane of the parathyroid glandular cells. Even small fluctuations in extracellular ionized calcium can generate intracellular signals via the G-protein complex that inhibit PTH release when blood calcium rises [7].

PTH is synthesized by the chief cells of the parathyroid gland as a pre-pro-peptide of 115 amino acids, but the signal fragment (25 amino acids) is cleaved as the nascent peptide enters the lumen of the endoplasmic reticulum. The resultant pro-peptide is subjected to further proteolysis (loss of another 6 amino acids) as it passes through the endoplasmic reticulum and matures in the secretory vesicles. There are generally two forms of secretory vesicles in parathyroid cells: one contains the active, full-length 84-amino acid PTH or PTH 1–84, while the other type contains both PTH 1–84 and proteases cathepsins B and H. Cathepsin B cleaves PTH 1–84 to PTH 37–84 and a mixture of NH_2-truncated fragments that are released under the control of calcium-sensing receptor in response to elevated blood calcium [8,37]. This occurs in conjunction with the inhibition of the secretion of PTH 1–84 from the other type of secretory vesicle, resulting in a reduction of circulatory PTH 1–84 and a concomitant increase in C-terminal fragment production.

PTH 1–84 has a very short half-life in the circulation ($t_{1/2}$ ~3 to 8 min) [9]. It is mostly degraded by the liver, generating a mixture of N-terminal, mid-molecule and C-terminal fragments. Unlike C-terminal fragments, Kupffer cell-generated N-terminal fragments are degraded rapidly. Since N-terminal PTH fragments are not produced and accumulated in any appreciable amount from parathyroid tissues, most (up to 88%) plasma PTH bioactivity resides with intact PTH 1–84 [10,37]. Renal clearance is also important in PTH metabolism, and relatively small reductions in renal function may be accompanied by significant increases in both the intact hormone and its fragments. Although it was once thought that PTH 1–84 was the only biologically active peptide secreted by the parathyroid glands, it is now apparent that some long C-terminal fragments e.g., PTH 7–84 have potentially significant inhibitory properties [11,12], presumably through specific C-terminal PTH receptor actions mentioned above. Nevertheless, removal of one or more amino acids from the amino-terminus of PTH 1–84 renders the hormone less active, and large NH_2-terminal truncated fragments (e.g., PTHs 7–84, 19–34, 37–84) are largely inactive as PTH1R agonists.

Measurements of PTH

Initially, diagnostic accuracy of PTH immunoassays was limited by the cross-detection of inactive C-terminal fragments. So-called ‘mid-molecule assays’ (first-generation) were supplemented by more expensive NH_2-terminal assays that tended to yield more accurate estimates of biologically active PTH, especially in patients with renal failure. Thus, two-site assays (second generation) were a significant advance and remain the methods most commonly used today [13]. Although it was initially thought that the detection antibody in the two-site assays targeted the key NH_2-terminal amino acids (PTH 1–5), it became clear that the PTH fragments containing amino acids between 7 and 84 were also immunoreactive and the epitopes have been localized to the stretch of residues between amino acid 7 and 34 [14]. Subsequently, assays that target the whole PTH 1–84 peptide (‘bio-intact PTH’, see Fig. 1) have been developed and made available commercially [15]. However, under physiological conditions and in chronic renal disease, the ratio of PTH 7–84 fragment and whole PTH 1–84 is roughly constant [16], although whole PTH 1–84 has been argued to provide better sensitivity for detecting primary hyperparathyroidism [17]. DOQI Guidelines from the National Kidney Foundation in the United States indicate that the advantages of these third-generation assays are insufficient to warrant wholesale replacement of the second-generation ones for the purpose of monitoring chronic kidney disease [18]. Although the results from second- and third-generation PTH assays generally correlate well and their normal reference ranges are reasonably comparable, the overall differences do not allow these results to be fully transferable numerically [19].

In Ontario, this problem is compounded by the use of different units of measurement. There are obvious reasons for adoption of molar units whenever possible, but this ideal is difficult or impossible to implement when there is microheterogeneity of the PTH molecular species. Currently, it is considered acceptable to use the calculated MW for PTH 1–84 in second-generation assays, so that the conversion of mass units in nanograms per liter (ng/L), or picograms per milliliter (pg/mL), to molar units (pmol/L) is a single factor of about 0.105.

As yet, there is no standardization sufficiently reliable to harmonize results among methods. By way of example, a recent comparison of 15 different assays (13 second- and third-generation assays) found that the median bias ranged from −44% to +123%, using the Nichols Allegro® intact-PTH assay as the reference [20]. Thus, clinical laboratories are dependent on