New lessons from old assays: parathyroid hormone (PTH), its receptors, and the potential biological relevance of PTH fragments

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Introduction

Accurate and reliable measurements of the concentration of parathyroid hormone (PTH) in serum or plasma are necessary for the diagnostic assessment of various clinical disorders of bone and mineral metabolism [1,2]. In patients with chronic renal failure, serum or plasma PTH levels are commonly used to identify different subtypes of renal osteodystrophy and to monitor evolution of the disorder [3–10]. Largely for practical reasons, plasma PTH levels serve as surrogates for bone histology in patients with end-stage renal disease (ESRD), and they are an essential guide to ongoing clinical management, particularly during the treatment of secondary hyperparathyroidism with vitamin D sterols [11–13].

It is now generally recognized that the several PTH assays that have been employed widely for many years do not measure exclusively the full-length biologically active hormone comprised of 84 amino acids, or PTH(1–84) [14–16]. Rather, these assays also detect large N-terminally truncated, PTH fragments that are present in plasma not only in patients with chronic renal failure but also in those with normal renal function [15,16]. Recent experimental evidence suggests that some of these peptides have biological actions in bone that are distinct from those traditionally ascribed to PTH(1–84) [17,18]. Such findings have important implications for the diagnosis and clinical management of patients with renal bone disease in particular and for understanding bone biology in general.

Secretion and metabolism of PTH

Apart from its effect to regulate PTH release from the parathyroid glands by modulating calcium-sensing receptor activity, variations in blood ionized calcium concentration also influence PTH metabolism [19–21]. It is generally thought that PTH is secreted predominantly as PTH(1–84), but the hormone is degraded within parathyroid cells and some of the resultant peptide fragments are released into peripheral blood. Relatively greater amounts of N-terminally truncated, or C-terminal, PTH fragments and lesser amounts of PTH(1–84) are detected in plasma when extracellular calcium concentrations rise [21–23]. In contrast, proportionately more PTH(1–84) and less of its C-terminal fragments are found when blood ionized calcium concentrations are reduced [22]. PTH is also metabolized in several peripheral tissues, including the liver [24,25].

PTH(1–84) and its various peptide fragments are removed from the circulation by glomerular filtration in the kidney [26]; they are subsequently degraded in cells of the proximal nephron, most likely after uptake by megalin-dependent mechanisms [27]. In patients with little or no renal function, the plasma clearance of PTH(1–84) and other PTH fragments is reduced, leading to the abundant accumulation of C-terminal peptides in peripheral blood [26].

Thus, alterations in the degradation of PTH(1–84) within the parathyroid glands, variations in the peripheral metabolism of PTH(1–84), and disturbances in the renal clearance of PTH(1–84) and other PTH-derived peptides can each potentially affect the relative amounts of PTH(1–84) and other peptide fragments in plasma. Because the N-terminal portion of PTH(1–84) mediates most of the classical actions of PTH by activating the type I PTH receptor, or PTHR1, N-terminally truncated PTH fragments have traditionally been thought to lack biological activity. Recent observations, however, challenge this view,
Assays for PTH

The presence of N-terminally truncated, or C-terminal, PTH fragments in plasma, particularly in patients with renal failure, has always presented a major technical challenge in the development of valid PTH assays. Those introduced during the 1960s and 1970s utilized conventional radioimmunoassay (RIA) methods [31,32]. Isotopically labelled peptides, usually highly purified intact PTH or synthetic PTH fragments, were employed in competitive displacement assays using single antibodies to detect the hormone in samples of serum or plasma. Most detection antibodies in RIAs for PTH were directed toward epitopes located within the mid- or C-terminal regions of the hormone due to the greater immunogenetic responses that were elicited by these portions of the molecule. As a result, virtually all RIAs for PTH detected a variety of C-terminal PTH fragments in addition to PTH(1–84). Because various C-terminal PTH fragments are retained in the plasma of patients with chronic renal failure, the results obtained using these PTH RIAs were difficult to interpret in those with end-stage renal disease (ESRD), and measurements were frequently unreliable and poorly reproducible. Values were often strikingly elevated but failed to consistently reflect the histological severity of hyperparathyroidism as documented by bone biopsy [33,34].

Many of the technical shortcomings of RIAs for PTH were overcome with the introduction of immunometric assays [35]. These systems utilize two antibodies in a ‘sandwich’-type assay to detect relatively longer peptides, presumably PTH(1–84) [35,36]. One antibody is directed toward an epitope located in the C-terminal region of PTH; it is attached to a solid phase, such as a plastic bead, and serves to capture the hormone in plasma of patients with chronic renal failure, the results obtained using these PTH RIAs were difficult to interpret in those with end-stage renal disease (ESRD), and measurements were frequently unreliable and poorly reproducible. Values were often strikingly elevated but failed to consistently reflect the histological severity of hyperparathyroidism as documented by bone biopsy [33,34].

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Fig. 1. A schematic depicting features of first- and second-generation immunometric assays for PTH and potential interactions between different portions of PTH(1–84) and its receptors. Both immunometric methods utilize capture antibodies directed toward epitopes located within the C-terminal region of PTH, whereas their detection antibodies target epitopes closer to the N-terminal end of the molecule. In first-generation immunometric PTH assays, detection antibodies are directed most probably to a region between amino acid residues 15 and 34. In second-generation immunometric assays, detection antibodies target epitopes within the most N-terminal portion of the molecule. Synthetic PTH peptides that are truncated at the N-terminal end such as PTH(2–34), PTH(3–34) and PTH(7–84) do not cross-react with the detection antibodies used in second-generation PTH assays. In contrast, these peptides interact with the detection antibodies employed in first-generation assays. The PTH1R binds to the most N-terminal portion of PTH; it is activated equally by PTH(1–84) and PTH(1–34) but does not bind to or respond to N-terminally truncated PTH fragments such as PTH(7–84). The C-PTH receptor binds to mid- and/or C-terminal portions of PTH. It can thus interact with N-terminally truncated PTH fragments such as PTH(7–84) and PTH(19–84) as well as with full-length PTH(1–84) but not with synthetic peptides that lack this portion of the molecule such as PTH(1–34).

Studies over the past several years provide compelling evidence that first-generation immunometric PTH assays not only detect PTH(1–84) but also cross-react with other large N-terminally truncated PTH fragments, some of which run on high-performance liquid chromatography at the same position as synthetic PTH(7–84) [14,37,38]. In contrast, recently introduced second-generation immunometric assays detect PTH(1–84) exclusively [15,16,18]. These new assays do not detect synthetic PTH(7–84) or other peptides lacking one or several of the N-terminal amino acid residues of PTH(1–84) [15] (Figure 1).

The specificity of second-generation immunometric PTH assays for PTH(1–84) is determined by detection antibodies directed toward epitopes located in the most N-terminal portion of the molecule. Indeed, removal of the first amino acid from synthetic PTH(1–34) eliminates its cross-reactivity with the detection antibody used in one second-generation immunometric assay [15]. In contrast, the detection antibodies in first-generation immunometric PTH assays bind to epitopes located further from the N-terminal end of the molecule, probably between amino acid residues 15 and 34. First-generation immunometric PTH assays thus detect both PTH(1–84) and large N-terminally truncated PTH fragments such as PTH(7–84) [15,16] (Figure 1).

When measured by second-generation immunometric assays, plasma PTH concentrations are approximately 40–50% lower than those determined using first-generation immunometric assays both in subjects...
with normal renal function and in patients with renal failure [15,16,18,28]. Divergences from this relationship occur, however, in some patients with ESRD, but the mechanisms responsible remain uncertain [15,28]. Overall, results obtained using first- and second-generation immunometric assays are highly correlated across a wide range of PTH concentrations both in patients with ESRD and in those with primary hyperparathyroidism [15,16,18].

**PTH-derived peptides and other PTH receptors**

Signal transduction through the PTH1R accounts for most of the classical biological actions of PTH [39]. Ligand binding to, and activation of, the PTH1R is mediated by highly specific interactions between the N-terminal domain of PTH(1–84) and several regions of the PTH1R, including the N-terminal extracellular domain, portions of some membrane-spanning helices, and extracellular loops [40,41]. Changes in the amino acid composition of the N-terminal portion of PTH(1–84) or truncations at the N-terminal end of the molecule markedly diminish or eliminate PTH1R activation [42].

Two other PTH receptors that differ significantly from PTH1R have been identified and cloned [43–45]. The PTH2 receptor, PTH2R, is expressed primarily in the central nervous system, pancreas, testis, and placenta. Unlike the PTH1R, the PTH2R from several species is activated only poorly or not at all by PTH and PTH-related protein, or PTHrP [46]. Only the human PTH2R is efficiently activated by PTH [43,47]. Recent data suggest that tuberoinfundibular protein (TIP39), a 39 amino acid peptide, is most probably the primary ligand for the PTH2R [48]. TIP39 was originally isolated from bovine hypothalamus and has some distant homology to both PTH and PTHrP [48,49]. A clear functional role for the PTH2R has yet to be established, but it may be involved in pain perception [50]. A third PTH receptor, PTH3R, has been identified thus far only in zebrafish [44,45]. Both PTH and PTHrP bind to and activate this telost receptor with similar high efficiency, but it is not activated by TIP39 [51].

Whereas PTH1R and other recently cloned PTH receptors bind to the N-terminal portion of PTH and PTHrP, several studies have strongly suggested the existence of a distinct receptor that interacts with mid- and/or C-terminal regions of PTH [52–56]. This putative receptor, which has yet to be cloned, has been tentatively designated as the C-PTH receptor. Its capacity to interact with N-terminally truncated PTH fragments suggests a mechanism to account for biological effects of peptide fragments that have heretofore been considered to be inactive byproducts of PTH metabolism.

High-affinity radioligand binding for recombinant analogues of human PTH such as [Yr34]hPTH(1–84) and [Yr34]hPTH(19–84) has been demonstrated in clonal osteoblast-like cells, whereas neither ligand bound with high affinity to LL-CPK-1 cells stably expressing the recombinant PTH1R [56]. High-affinity radioligand binding to ROS 17/2.8 osteoblast-like cells and to osteocytic cells was dose-dependently diminished by unlabelled PTH(1–84) and by several C-terminal fragments of PTH(1–84) but not by synthetic PTH(1–34) [17,56]. Thus, both osteoblasts and osteocytes appear to have a distinct receptor that interacts preferentially with the mid- or C-terminal regions of PTH.

Signalling through the putative C-PTH receptor has been reported to modulate bone cell activity both in vivo and in vitro. In thyro-parathyroidectomized rats, the i.v. or i.p. administration of synthetic PTH(7–84) and other N-terminally truncated PTH peptides counteract the effect of PTH(1–84) and PTH(1–34) to raise blood ionized calcium concentrations [17,18]. Because PTH(7–84) does not bind efficiently to the PTH1R and does not elicit a cAMP response in osteoblast-like cells, the effect of PTH(7–84) to offset the in vivo calcaemic response to both PTH(1–84) and PTH(1–34) is probably mediated through the C-PTH receptor [17]. The additional finding that cAMP generation in ROS 17/2.8 cells is greater during incubations with PTH(1–34) than with equimolar concentrations of PTH(1–84) is consistent with such a mechanism [17].

Studies by Divieti et al. [29,30] indicate that human PTH(7–84) diminishes 45Ca release from pre-labelled neonatal mouse calvariae and reduces in vitro bone resorption that has been activated by several agents including human PTH(1–84) and PTH(1–34). Exposure to synthetic human PTH(7–84) and PTH(39–84) also decreased the formation of osteoclast-like cells in murine bone marrow cultures induced by 1,25-dihydroxyvitamin D3 [29]. Such findings suggest that N-terminally truncated PTH fragments modify the recruitment and/or differentiation of osteoclasts and diminish bone resorption, probably by signalling through the C-PTH receptor in osteoblasts and/or osteocytes [17,30].

**PTH and the regulation of bone metabolism in renal failure**

The results from recent experimental studies are directly and immediately relevant to disturbances in bone metabolism due to chronic renal failure. One important issue pertains to PTH measurements and their role in the clinical management of patients with ESRD. The potential inhibitory effects of N-terminally truncated PTH fragments on bone cells have broad implications for the modulation of bone metabolism and calcium homeostasis in general, but they are also germane to the regulation of bone remodelling and turnover in ESRD.

As noted previously, plasma PTH levels obtained using second-generation immunometric assays are
substantially lower than those determined using first-generation assays. As such, clinicians will need to carefully interpret plasma PTH values based upon the type of immunometric PTH assay utilized. Current guidelines about the diagnosis and management of renal osteodystrophy are based almost exclusively upon results obtained using first-generation immunometric PTH assays [3–10], whereas only limited information is available describing the relationship between bone histology and plasma PTH levels using second-generation immunometric PTH assays [28].

The accumulation of substantial bone histology data in patients with ESRD is needed to determine whether second-generation assays are superior to first-generation assays as a biochemical indicator of renal bone disease. To date, there is insufficient information about bone histology to guide diagnostic and therapeutic decisions using second-generation immunometric PTH assays in patients with ESRD. Definitive recommendations about second-generation immunometric PTH assays and their utility for establishing the diagnosis of renal osteodystrophy and for monitoring progression of the disorder must await thorough histological assessments of bone in diabetic and non-diabetic subjects with chronic renal failure, in patients who have not been treated with vitamin D sterols, and in those given either small daily oral doses or larger intermittent parenteral doses of vitamin D. Until such information becomes available, the meaning of results from second-generation immunometric PTH assays in patients with ESRD can only be inferred by understanding their relationship to first-generation assays that have been validated by adequate bone histomorphometry data.

The putative inhibitory effects of N-terminally truncated PTH fragments such as PTH(7–84) on bone metabolism offer interesting new insights into mechanisms that could account for skeletal resistance to PTH in chronic renal failure. To address this issue, it will be necessary to biochemically define these N-terminally truncated PTH fragments, to more fully characterize the roles of PTH(7–84) and other large N-terminally truncated PTH fragments as activators of the C-PTH receptor, and to determine whether and how these peptides modify signal transduction through the PTH1R. Alterations in the relative abundance of PTH(1–84) and various PTH-derived peptides have been reported to adversely affect bone turnover in patients with ESRD, and it has been suggested that estimates of the relative concentrations of N-terminally truncated PTH fragments and PTH(1–84) are a useful predictor of adynamic renal osteodystrophy in patients with ESRD [28]. A number of methodological issues must be resolved, however, and additional confirmatory data are needed to adequately document a role for these peptides as key determinants of bone remodelling in patients with renal bone disease.

In summary, the availability of highly specific assays for PTH(1–84) should make it possible to more precisely define the role of PTH as a regulator of bone formation and turnover in chronic renal failure. The ability to estimate, or perhaps in the future to selectively measure, the concentration of various N-terminally truncated PTH fragments in serum or plasma provides additional opportunities to explore the potential functional role(s) of these molecules in the regulation of bone metabolism.

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