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PERSPECTIVES IN RENAL MEDICINE

Parathyroid hormone (PTH), PTH-derived peptides, and new PTH assays in renal osteodystrophy

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Parathyroid hormone (PTH), PTH-derived peptides, and new PTH assays in renal osteodystrophy. Reliable measurements of parathyroid hormone (PTH) concentrations in serum or plasma are critical for the appropriate diagnosis and management of patients with renal osteodystrophy. With the introduction of second generation immunometric assays for PTH, it is now possible to measure exclusively full-length, biologically active PTH(1-84). In contrast, first generation immunometric assays that have been used widely for many years detect not only PTH(1-84), but also other large amino-terminally-truncated, PTH-derived peptides. This development will require a careful re-evaluation of PTH measurements, as determined by either first or second generation immunometric assays, and their relationship to bone histology and bone remodeling rates in patients with end-stage renal disease (ESRD). Such information is essential for proper clinical management, but only limited bone biopsy data are available to guide the interpretation of PTH results using second generation PTH assays. The different performance characteristics of first and second generation immunometic PTH assays also makes it possible to quantify the plasma levels of amino-terminally-truncated, PTH-derived peptides, which may accumulate disproportionately in patients with ESRD. Recent experimental evidence indicates that one or more of these peptides can modify bone cell activity and skeletal remodeling, possibly by interacting with a PTH receptor distinct from the type I PTH receptor that binds to the amino-terminal portion of PTH and mediates the classical biological actions of the hormone. The putative C-PTH receptor interacts with mid- and/or carboxyterminal regions of PTH and other amino-terminally-truncated PTH-derived peptides; signaling through it may contribute to the skeletal resistance to PTH that characterizes ESRD. The current review discusses certain aspects of the molecular structure of PTH and its interaction with various receptors, briefly comments about selected components of PTH secretion, highlights recent technical advances in PTH assays, and summarizes the effects of various PTH-derived peptides on bone cells and on skeletal metabolism.

Key words: immunometric PTH assay, clinical management, end-stage renal disease, peptides and bone cells, chronic renal failure, skeletal lesions, PTH(1-84), renal bone disease.

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Reliable measurements of the concentration of parathyroid hormone (PTH) in serum or plasma are essential for the effective clinical management of patients with chronic renal failure. Metabolic bone disease is common in such patients [1, 2], and most available evidence indicates that PTH is the major determinant of the rates of bone remodeling and turnover in those with end-stage renal disease (ESRD) [3]. Plasma PTH concentrations, which predominantly reflect basal rates of PTH release from the parathyroid glands [4], largely determine whether patients with ESRD develop high-turnover skeletal lesions of renal osteodystrophy due to secondary hyperparathyroidism or low-turnover lesions such as adynamic bone [1, 2, 5, 6]. Accurate assessments of plasma PTH levels thus are essential not only for the proper laboratory diagnosis of renal bone disease but also to appropriately monitor evolution of the disorder, particularly during the treatment of secondary hyperparathyroidism with active vitamin D sterols [2].

Several developments in recent years have served to challenge prevailing concepts about the value of PTH measurements in patients undergoing regular dialysis. Among these was the observation that bone formation, as measured by the technique of double-tetracycline labeling in iliac crest bone biopsies, decreased dramatically in some patients with secondary hyperparathyroidism after treatment with large intermittent doses of calcitriol, or 1,25-dihydroxyvitamin D_3 [7, 8]. Such findings suggested that plasma PTH levels may not accurately reflect bone formation and turnover, and ultimately bone histology, during intermittent calcitriol therapy. Similar changes also might occur during treatment with new vitamin D sterols such as paricalcitol (Zemplar®) or doxercalciferol (Hectorol®), but data that address this issue have yet to be reported.

Other work has questioned the validity of serum or plasma PTH measurements as a reliable biochemical indicator of the underlying type of renal bone disease in patients with ESRD [9]. Different criteria for the inclu-

sion of study participants and methodological issues probably account for certain disparities among published reports. It should be recognized, however, that much of the information that documents the relationship between serum or plasma PTH levels and bone histology in patients with ESRD was collected more than 15 years ago [1, 6, 10–14]. Although the results continue to guide current diagnostic and therapeutic decisions, the data were obtained when therapeutic approaches to secondary hyperparathyroidism differed substantially from those employed currently. They largely represent findings in untreated patients and those managed with small daily oral doses of calcitriol, whereas large thrice-weekly intravenous doses of calcitriol and other vitamin D sterols now are widely used to treat secondary hyperparathyroidism [15–18]. Moreover, many of the patients in older studies received aluminum-containing, phosphate-binding medications rather than large oral doses of calcium. Intermittent calcitriol therapy and high oral calcium intakes have each been cited as causes for the evolving histological pattern of renal bone disease in recent years and for the relatively higher prevalence of adynamic renal osteodystrophy in the contemporary dialysis population [1, 2, 19].

Beyond these historical developments, new PTH assays have been introduced, and it is now generally recognized that several widely utilized PTH assays cross-react with and detect peptide fragments that are distinct from full length, biologically active PTH, or PTH(1-84) (vide infra) [20–23]. Awareness of these technical advances has been spread through scientific meetings and by commercial endorsements. Certain proprietary claims appear premature, however, and have not been documented adequately. In this regard, it has been suggested that measurements of the concentration of amino-terminally-truncated PTH-derived peptides, or other PTH fragments, in serum or plasma are useful for the diagnosis of renal bone disease [24]. This issue deserves careful and objective scrutiny.

These striking developments have generated uncertainty and considerable confusion among clinicians about the pathophysiology, diagnosis, and appropriate management of renal bone disease. In this context, it should be recognized that few studies have been done using new PTH assays to assess disorders of bone and mineral metabolism in patients with ESRD, and there is a paucity of data to guide the interpretation of results. Indeed, only limited information is available about the relationship between plasma PTH levels, as determined by any of several new PTH assays, and the various subtypes of renal osteodystrophy, as documented by bone biopsy (abstract; Salusky et al, *J Am Soc Nephrol* 12:772A, 2001) [24]. It is not yet therefore known whether the information provided by new PTH assays will prove to be superior to that available using current assay methods either for the initial laboratory diagnosis of renal osteodystrophy or for monitoring progression of the disorder in patients with ESRD.

Recent work aimed at defining the molecular mechanisms responsible for the biological actions of PTH and other PTH-related peptides in bone and other target tissues provides important new information that is relevant to understanding bone metabolism and skeletal remodeling in chronic renal failure. The goal of the current discussion is to review various issues pertaining to the molecular structure of PTH and its interactions with various receptors, to briefly discuss selected components of PTH secretion, to summarize technical advances in methods for determining the concentration of PTH in biological samples, and to examine the physiological effects of various PTH-derived peptides on bone cells and their potential role as modifiers of skeletal metabolism. The integration of this information into current knowledge about bone and mineral metabolism in chronic renal failure may provide a broader fundamental understanding of renal bone disease.

THE MOLECULAR STRUCTURE OF PTH AND PTH RECEPTORS

Parathyroid hormone (PTH) is a peptide comprised of 84 amino acids [25, 26]. It initiates signal transduction in a variety of tissues by binding to the type I PTH (PTH1) receptor, accounting for most of the classical biological actions of PTH [27]. The PTH1 receptor also is known as the common receptor for PTH and PTH-related peptide, or PTHrP, because it binds to and initiates signal transduction events in response to both PTH and PTHrP (Fig. 1) [27].

The binding of PTH to its receptor is mediated by the amino-terminal, or N-terminal, portion of the peptide [28]. This is also the case for PTHrP, a larger peptide hormone that appears as three isoforms comprised of 139, 141 or 171 amino acids [29]. Excess production of PTHrP by various tumors is usually responsible for the syndrome of humoral hypercalcemia of malignancy [29].

The N-terminal portions of PTH and PTHrP are identical for eight of their first 13 N-terminal amino acid residues [29], and the two peptides are equivalent in activating the PTH1 receptor [27]. Recent efforts have documented the importance of several key sites within the first 34 N-terminal amino acids of PTH that are involved in high-affinity interactions with the PTH1 receptor, some of which are crucial for receptor activation, probably by mediating conformational changes in the receptor protein. Thus, PTH binds to both the extracellular, N-terminal domain and to extracellular loops within the membrane spanning portion of the PTH1 receptor [30, 31]. Modifications in the amino acid composition of the N-terminal portion of PTH(1-84) or deletions at the amino-terminus of the molecule substantially diminish

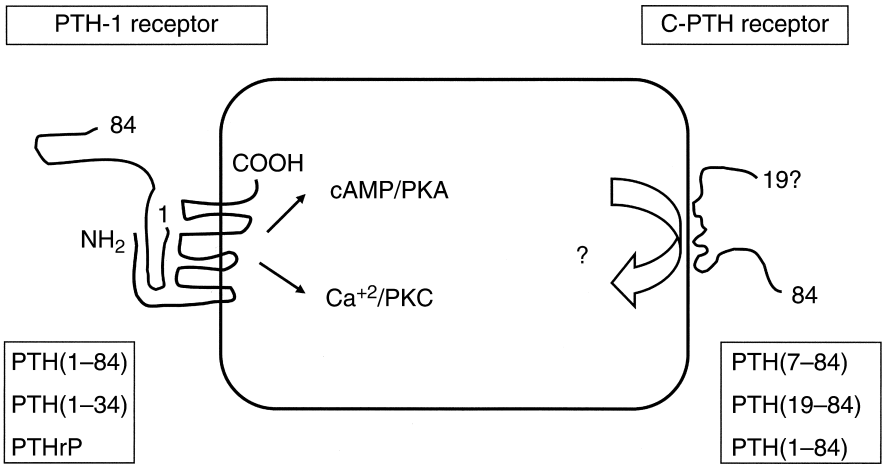

Fig. 1. Depiction of two distinct cellular receptors for parathyroid hormone (PTH). The PTH1 receptor mediates most of the classical biological actions of full-length PTH(1-84) in target tissues by initiating intracellular signal transduction through protein kinase A and protein kinase C. The amino-terminal domains of both PTH and parathyroid hormone-related peptide (PTHrP) bind with equal affinity to the PTH1 receptor. In contrast, the putative C-PTH receptor interacts with portions of PTH that are located further toward the mid- and carboxy-terminal regions of the peptide. The C-PTH receptor binds to several amino terminally truncated PTH-derived peptides including PTH (7-84), but signal transduction pathways for the C-PTH receptor have yet to be characterized.

binding to the PTH1 receptor [28]. In contrast, truncations at the carboxy-terminal, or C-terminal, end of PTH (1-84) do not affect receptor affinity. Therefore, both PTH(1-34) and PTH(1-84) exhibit equal ligand binding affinity for the PTH1 receptor (Fig. 1).

In addition to the PTH1 receptor, two other PTH receptors have been identified and cloned. The PTH2 receptor was originally isolated from cDNA libraries from cerebral cortex [32]. It is expressed predominantly in the central nervous system, pancreas, testis and placenta. The PTH2 receptor appears to be more ligand-specific than the PTH1 receptor. Whereas the N terminal domains of PTH and PTHrP both bind to and initiate signal transduction through the PTH1 receptor, PTH2 receptors from several species are activated only poorly or not at all by PTH or PTHrP [33]. Only the human PTH2 receptor is efficiently activated by PTH [32, 34]. Recent studies suggest that a 39 amino-acid peptide, tuberoinfundibular protein (TIP39), is most likely the natural ligand for the PTH2 receptor [35]. This peptide was initially isolated from bovine hypothalamus and shows some distant homology to PTH and PTHrP [35, 36]. Interestingly, TIP39 and truncated analogs of it can bind to the PTH1 receptor, raising the possibility that it may act as an inhibitor of PTH or PTHrP actions [37, 38]. The functional role(s) of the PTH2 receptor remain uncertain, but there is evidence that it may be involved in pain perception [39]. A third receptor, the PTH3 receptor, has been identified only in zebrafish [40, 41]. PTH and PTHrP each bind with similarly high efficiency to the zebrafish PTH3 receptor, but it is not activated by TIP39 [42].

More pertinent to recent discussions about the biological actions of PTH in renal failure are data reported originally many years ago but that have been characterized in greater detail more recently to suggest the existence of a distinct PTH receptor that interacts with mid- and/or C-terminal regions of PTH [43–47]. In recent discussions, this putative receptor, which has yet to be cloned, has been called the C-PTH receptor (Fig. 1). In clonal osteoblast-like cells, high affinity radioligand binding could be demonstrated for recombinant analogs of human PTH such as [Tyr34]hPTH(1-84) and [Tyr34]hPTH (19-84), whereas neither ligand bound with high affinity to LLCPK1 cells that stably expressed the recombinant PTH1 receptor [47]. In addition, high-affinity radioligand binding to ROS17/2.8 osteoblast-like cells and to osteocytic cells was reduced by the addition of increasing amounts of several unlabeled C-terminal fragments of PTH(1-84), but not by synthetic PTH(1-34) [47]. Such findings provide firm experimental evidence that bone cells of the osteoblastic lineage have a distinct PTH receptor that interacts with the mid- or carboxy-terminal regions of PTH.

The potential functional relevance of the C-PTH receptor as a modulator of bone cell activity has been examined both in vivo and in vitro. Nguyen-Yamamoto and co-workers reported that intravenous infusions of synthetic PTH(7-84) and other N-terminally-truncated PTH peptides, including PTH(39-84) and PTH(53-84), counteract the effects of PTH(1-84) and PTH(1-34) to raise blood ionized calcium concentrations in thyro-parathyroidectomized rats [48]. Qualitatively similar, but less complete, results were presented earlier by Slatopolsky et al [49]. Nguyen-Yamamoto and co-workers also demonstrated that synthetic PTH(1-84) and PTH(7-84) both bind specifically to the putative C-PTH receptor, whereas PTH(1-34) does not, findings consistent with earlier experimental observations [48]. Conversely, both PTH(1-84) and PTH(1-34) were shown to bind to the PTH1 receptor and increase cyclic adenosine 3′,5′-monophosphate AMP (cAMP) levels in ROS 17/2.8 cells, whereas PTH(7-84) failed to effectively compete for binding to the PTH1 receptor or generate a cAMP response. Interestingly, cAMP generation was greater during incubations with PTH

(1-34) than with PTH(1-84) at equimolar concentrations in ROS 17/2.8 cells [48]. These latter results are consistent with the view that binding of the mid- or C-terminal portions of PTH(1-84) to the C-PTH receptor modulates, or partially offsets, the agonist actions of PTH(1-84) that are mediated through the PTH1 receptor (Fig. 1).

Divieti et al provided additional evidence that signaling through the C-PTH receptor can influence bone metabolism in vitro [50]. Incubations with human PTH(7-84) diminished ^{45}Ca release from pre-labeled neonatal mouse calvariae and also reduced in vitro bone resorption induced by several agents including human PTH(1-84), PTH(1-34), 1,25-dihydroxyvitamin D_3, prostaglandin E_2 (PGE_2), and interleukin-11 (IL-11). Moreover, both synthetic human PTH(7-84) and PTH(39-84) impaired the formation of osteoclast-like cells in response to 1,25-dihydroxyvitamin D_3 in murine bone marrow cultures [50]. Because osteoclastic bone resorption that is initiated by agents such as PTH, 1,25-dihydroxyvitamin D_3, PGE_2 and IL-11 is modulated by RANKL and osteoprotegerin (OPG) [51], it is plausible that the inhibitory actions of PTH analogs such as PTH(7-84) or PTH(39-84) on osteoclastogenesis are mediated by variations in RANKL or OPG expression. Direct effects of C-terminal PTH fragments on osteoclasts or osteoclast-precursors cannot, however, be excluded. Thus, N-terminally-truncated PTH-derived peptides appear not only to influence osteoblastic function but also to affect osteoclastic differentiation and bone resorption by signaling through a C-PTH receptor. High levels of C-PTH receptor expression in osteocytes in vitro suggest an additional cellular target for N-terminally-truncated PTH-derived peptides in bone [52].

The existence of a C-PTH receptor suggests a potential mechanism to account for recent observations in several experimental models (Fig. 1). Considerable additional work will be required, however, to adequately document the clinical relevance of these findings to the regulation of skeletal metabolism in humans with renal osteodystrophy.

CALCIUM-MEDIATED PTH SECRETION AND ITS EFFECT ON THE SPECIES OF PTH IN PERIPHERAL BLOOD

It is generally thought that PTH is released from parathyroid cells predominantly as the full length biologically active hormone, PTH(1-84). PTH that is stored within secretory granules can be degraded, however, within the cell, particularly in the presence of high extracellular calcium concentrations, and some of these peptide fragments gain access to the circulation [53, 54]. PTH(1-84) also undergoes metabolism in peripheral tissues, most notably liver [55]. Thus, PTH-derived peptides other than PTH(1-84) are present in peripheral blood [20, 56].

The relative amounts of PTH(1-84) and other PTH-derived peptides in serum or plasma are affected by variations in blood calcium levels. At reduced blood ionized calcium concentrations, PTH(1-84) appears to be the predominant form of PTH secreted by the parathyroid glands and is the major species of PTH found in peripheral blood when measured using assays that detect the N terminal portion of the molecule; lesser amounts of N-terminally-truncated, PTH-derived peptides, or carboxy-terminal PTH fragments, are detected [54, 56]. In contrast, relatively greater amounts of C-terminal PTH fragments are present in the circulation, as assessed by assay systems that cross-react with carboxy-terminal portions of the PTH molecule, when blood ionized calcium concentrations are elevated [56]. It remains uncertain whether these changes reflect variations in the metabolism of PTH within parathyroid cells, alterations in the degradation of PTH in peripheral tissues, or the aggregate result of both processes. Although calcium-regulated PTH secretion by parathyroid cells is mediated by the calcium-sensing receptor (CaSR) [57], the role of CaSR activation as a modifier of intracellular PTH degradation is not known. Similarly, treatment with vitamin D diminishes pre-pro-PTH gene transcription [58, 59], but little is known about direct effects of vitamin D on PTH metabolism. Vitamin D-mediated increases in serum calcium concentrations would be expected, however, to influence the metabolism of PTH as discussed previously.

The release of abundant amounts of full length, biologically active PTH(1-84), and lesser amounts of C-terminal PTH fragments from the parathyroid glands, under conditions of hypocalcemia, theoretically, would maximize biological responses mediated by PTH1 receptors located in kidney and bone, serving to restore serum calcium concentrations to normal. Conversely, the release of relatively greater amounts of C-terminal PTH fragments, as PTH(1-84) levels decline under conditions of hypercalcemia, could confer an additional level of regulatory control over serum calcium concentrations if N-terminally-truncated PTH-derived peptides such as PTH(7-84) or PTH(19-84) indeed modulate the agonist actions of PTH(1-84) [48, 49]. Although preliminary data support this possibility, formal experimental confirmation is needed to determine whether variations in the species of PTH in the circulation participate in the regulation of serum calcium concentrations by influencing calcium fluxes in kidney and/or bone.

METHODS FOR MEASURING THE CONCENTRATION OF PTH IN SERUM OR PLASMA

Adequate control of secondary hyperparathyroidism is important in managing bone disease in patients with ESRD. Histopathology is the definitive method for documenting various forms of renal osteodystrophy, but bone biopsy is an invasive diagnostic procedure and relatively

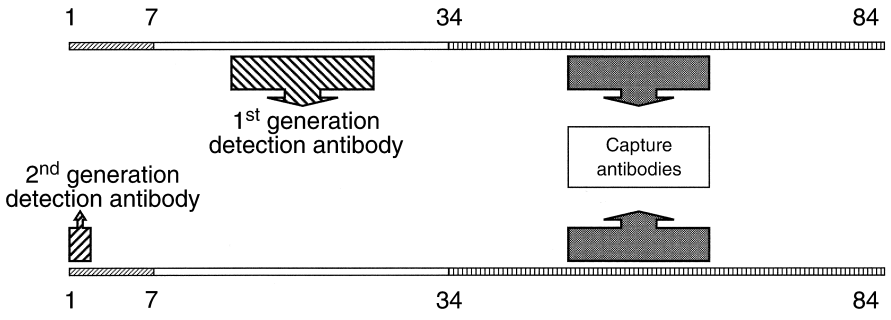

Fig. 2. A comparison of technical aspects of first and second generation immunometric assays for PTH. Both methods employ capture antibodies directed toward epitopes located within the carboxyterminal region of the peptide to immobilize labeled hormone on a solid phase. Detection antibodies are targeted to epitopes located closer to the amino-terminal end of the peptide. In first generation immunometric PTH assays, detection antibodies were directed, most probably, to a region between amino acid residues 15 and 34. By contrast, detection antibodies in second generation immunometric PTH assays are directed toward epitopes in the most amino-terminal portion of the molecule. Synthetic PTH peptides that are truncated at their amino-terminal end such as PTH(2-34), PTH(3-34) and PTH(7-84) are not detected in second generation assays, but cross-react with the detection antibodies used in first generation assays.

few laboratories have the expertise and equipment needed to do bone histomorphometry. Thus, clinicians rely almost exclusively upon plasma or serum PTH determinations to guide clinical management.

Assays for PTH that were developed during the 1960s, 1970s and 1980s employed conventional radioimmunoassay (RIA) techniques [60, 61]. Here, isotopically labeled peptides, usually highly purified intact PTH or synthetic PTH fragments, were used in competitive displacement assay systems to measure hormone concentrations in serum or plasma. Because the most antigenic portions of PTH are located in the mid- and carboxy-terminal regions of the molecule, virtually all RIAs for PTH detected a variety of carboxy-terminal PTH fragments in addition to full-length, biologically active PTH(1-84). These features posed serious problems for the interpretation of results in patients with chronic renal failure.

Parathyroid hormone and various of its peptide fragments are largely cleared from the circulation by glomerular filtration, and they undergo catabolism within tubular epithelial cells of the proximal nephron after uptake via megalin-dependent mechanisms [62, 63]. In patients with end-stage renal disease, fragments of PTH accumulate in plasma and are found in high concentrations. Since many of these fragments cross-reacted with antibodies used in various PTH RIAs, serum PTH values determined by these assays were frequently markedly elevated in patients with chronic renal failure, measurements were often unreliable, and results corresponded poorly to histological findings documented by bone biopsy [12, 64].

Many of the technical shortcomings of RIAs for PTH were overcome with the introduction of immunometric assays [65], which were originally called immunoradiometric assays, or IRMAs, because they used a radioisotope to label detection antibodies. These systems employ two antibodies in a "sandwich" type assay to detect longer peptides, presumably full length intact PTH(1-84), minimizing cross-reactivity with smaller PTH fragments. Technically, one antibody, which is directed toward an epitope located in the C-terminal region of PTH, is attached to a solid phase, such as a plastic bead, and serves to capture the hormone in serum or plasma (Fig. 2). A second antibody, directed at an epitope within the N-terminal region of PTH, is labeled, usually with ^{125}I or a chemiluminescent agent, and serves as the signaling antibody (Fig. 2). Aliquots of serum or plasma containing unknown concentrations of PTH are incubated with both antibodies, and the amount of the signaling antibody that separates with the solid phase is then measured. Relatively small peptide fragments that bind to either antibody alone are not detected in these assay systems because a relatively long peptide is required to bridge the space between the two epitopes located in different regions of the PTH molecule (Fig. 2). Only peptides of sufficient length to bridge both epitopes within PTH and thereby link the immobilized antibody to the labeling antibody are detected.

Until recently, first generation immunometric PTH assays were thought to detect either predominantly or exclusively full length PTH(1-84). It is now evident, however, that this is not the case. First generation immunometric PTH assays cross-react with other large amino-terminally-truncated PTH-derived peptides, some of which co-elute on high-performance liquid chromatography (HPLC) with synthetic PTH(7-84) [20, 21]. It is likely that other PTH-derived peptides also are present in serum or plasma, particularly in patients with ESRD. By contrast, recently introduced second generation immunometric PTH assays detect PTH(1-84) exclusively [22, 23, 49]. They do not cross-react with synthetic PTH(7-84) or other peptide fragments that lack the first few N-terminal amino acids of PTH(1-84) (Fig. 2) [22].

For second generation immunometric PTH assays, specificity for PTH(1-84) is determined by a labeling antibody that is directed toward an epitope located within the most N-terminal portion of the molecule. Synthetic peptides that have been truncated at the N terminal end such as PTH(7-84) are not detected because the epitope is either absent or structurally altered. Indeed, deletion of only the first amino acid from synthetic PTH(1-34) is sufficient to eliminate cross-reactivity of the peptide with the detection antibody used in certain second generation immunometric PTH assays [22]. In contrast, detection antibodies used in first generation immunometric PTH assays were directed toward epitopes located more distally within the amino-terminal portion of the PTH molecule, probably between amino acid residues 15 and 34. As such, first generation immunometric PTH assays were fully capable of detecting N-terminally-truncated PTH fragments such as PTH(7-84) as well as full length PTH(1-84) (Fig. 2) [22, 23].

When measured by second generation immunometric PTH assays, plasma PTH concentrations are approximately 40 to 50% lower than values obtained using first generation immunometric assays both in subjects with normal renal function and in those with end-stage renal disease [22–24, 49]. Despite such differences, several studies, some of which have been published, demonstrate a high degree of correlation across a wide range of PTH concentrations between the results obtained with first and second generation immunometric PTH assays when measurements are done using both methods in the same plasma samples [22, 23].

PTH AND BONE METABOLISM IN RENAL FAILURE

As noted previously, virtually all of the available data about the relationship between plasma PTH levels and bone histopathology in patients with renal osteodystrophy have been generated using first generation immunometric PTH assays or older RIAs. Values determined by first generation immunometric PTH assays generally predict the underling type of renal bone disease in untreated patients and in those receiving small daily oral doses of vitamin D, specifically calcitriol [1, 14, 19, 66–68]. Published results are somewhat dated, however, and they may not accurately reflect the impact of current therapeutic practices, which often include the use of large, intermittent intravenous doses of vitamin D sterols and large oral doses of calcium. Both interventions appear to disrupt the relationship between plasma PTH levels and bone turnover, perhaps through direct inhibitory effects on osteoblasts [7, 8, 69–71]. Although some reports have questioned the diagnostic utility of first generation immunometric PTH assays to assess renal osteodystrophy in patients with ESRD, the results reflect data obtained from a rather heterogeneous population that included both hemodialysis and peritoneal dialysis patients, those given daily oral doses or intravenous doses of calcitriol, and untreated patients [9].

Bone biopsy information is just now beginning to be collected in ESRD patients who have been assessed using second generation immunometric PTH assays (abstract; Salusky et al, *ibid*) [24]. Considerable additional work is needed to establish the relationship between plasma PTH levels, as measured by second generation immunometric PTH assays, and the various subtypes of renal osteodystrophy not only in untreated patients, but also in those who are treated with active vitamin D sterols. To date, there is insufficient information to guide diagnostic and therapeutic decisions about renal osteodystrophy using new PTH assays due to the paucity of bone histology data.

In this regard, a recent report presents bone histomorphometric data and corresponding PTH results using both first and second generation immunometric PTH assays in 51 patients with ESRD [24]. The relationships between plasma PTH levels and bone remodeling, as measured by the histomorphometric parameter activation frequency, were similar with each assay. Neither assay was superior to the other in distinguishing between patients with adynamic renal osteodystrophy and those with normal or high rates of bone turnover as documented by bone biopsy [24].

However, the authors took advantage of technical differences between the two assays to estimate the concentration of N terminally-truncated PTH-derived peptide fragments in plasma. Based upon in vitro and in vivo experimental work mentioned previously, there is evidence to suggest that one or more N terminally-truncated, PTH-derived peptides exert inhibitory effects on osteoblasts and on bone remodeling. To address this issue, the concentration of PTH fragments [PTH(frag)] in plasma was estimated by subtracting PTH values determined using a second generation immunometric PTH assay, which detects PTH(1-84) exclusively, from values obtained using a first generation immunometric PTH assay that detects both PTH(1-84) and other relatively large N terminally-truncated PTH-derived fragments. A ratio depicting the relative abundance of biologically active PTH(1-84) to the amount of PTH fragments also was calculated and expressed as PTH(1-84)/PTH(frag) [24].

Overall, patients with adynamic renal osteodystrophy had lower PTH(1-84)/PTH(frag) ratios than those with either normal or high rates of bone turnover, and none of the patients with adynamic bone had a PTH(1-84)/PTH(frag) ratio that exceeded unity. Thus, there appears to be a relative abundance of N terminally-truncated PTH fragments compared to PTH(1-84) in patients with ESRD who have subnormal rates of bone turnover. It should be noted, however, that some patients with hyperparathyroid bone disease also had PTH(1-84)/PTH(frag)

ratios <1. As such, a calculated PTH(1-84)/PTH(frag) ratio <1 was not diagnostic of adynamic bone [24]. Nevertheless, the results were taken as evidence that N terminally-truncated, PTH-derived peptides have inhibitory effects on bone remodeling in patients with ESRD and that their accumulation in plasma accounts for the development of adynamic renal osteodystrophy.

The hypothesis set forth by these investigators is an interesting one, and it certainly warrants further investigation considering recent experimental observations. If documented adequately in future studies, information about factors that influence the generation and relative abundance of N terminally-truncated, PTH-derived peptides could offer additional insight into mechanisms that modulate osteoblastic activity and skeletal remodeling in chronic renal failure. Several issues must be addressed, however, in evaluating the clinical relevance of these findings.

One concern relates to apparent discrepancies with earlier reports about the relationship between PTH values obtained by two distinct immunometric PTH assays. In previous work, a high degree of correlation ($r = 0.977$) was shown between results obtained with the second generation immunometric PTH assay employed in the work by Monier-Faugere et al [24] and values determined using a widely utilized first generation immunometric PTH assay in 318 patients with ESRD [23]. Similarly high coefficients of correlation have been found in other comparative studies of first and second generation immunometric PTH assays, documenting a relatively fixed linear relationship between values obtained with each method (abstract; Salusky et al, *ibid*) [49]. Because these correlations are linear, there is a constant relationship, or ratio, as defined by the slope of the regression line, between PTH values measured by the two methods. Moreover, since the concentration of N terminally-truncated, PTH-derived peptides represents the simple numeric difference between two highly correlated variables, the PTH (1-84)/PTH(frag) ratio also, by definition, must remain fairly constant across the observed range of plasma PTH concentrations. In this context, it is difficult to understand the wide variation in values for the PTH(1-84)/PTH(frag) ratio reported in patients with ESRD, which ranged from 0.01 to 14.2 [24].

The use of activation frequency as the sole histomorphometric index of bone remodeling in patients with ESRD presents additional problems [24]. Most clinicians are unfamiliar with the term, how it is determined, and what it means. Measurements of bone formation are more familiar to a greater number of readers and they are better understood. Although not strictly a measure of bone remodeling, bone formation provides a concise objective measure of one component of the tightly-coupled process of bone remodeling. Information about bone formation also would facilitate comparisons with previously reported data from other bone histology laboratories in patients with renal bone disease [24]. Recent observations indicate that activation frequency does not offer additional information beyond that provided by measurements of bone formation, and there are methodological issues with determining activation frequency when bone formation rates are markedly reduced as in adynamic renal osteodystrophy [72]. Additional assessments using conventional measures of bone formation would be useful in future clinical studies to examine the putative inhibitory role of various PTH-derived peptides in renal bone disease.

Apart from technical issues, matters of patient selection constrain the broad application of recent findings. Although PTH is considered to be the major determinant of bone formation and turnover in ESRD, other factors also play a role [3]. Treatment with corticosteroids and aluminum intoxication each diminish bone formation and turnover, whereas growth hormone administration not only promotes linear growth, but also increases bone formation during skeletal remodeling [2, 73–75]. Diabetes mellitus can affect bone and mineral metabolism adversely even when renal function remains normal [76]. Osteoblastic activity is reduced and bone formation is subnormal in many diabetic subjects, and studies in experimental animals with diabetes indicate that insulin deficiency is largely responsible for these disturbances, which are not explained adequately by decreases in plasma PTH levels [77, 78].

Such observations in diabetic subjects are relevant to the current discussion of adynamic renal osteodystrophy because about 25% of the adult patients with ESRD who were evaluated by bone biopsy and concurrent estimates of the PTH(1-84)/PTH(frag) ratio had diabetes [24]. As such, a mechanism distinct from variations in the PTH(1-84)/PTH(frag) ratio could account for the presence of adynamic bone in many of the study participants. A homogenous group of patients unaffected by factors capable of independently modifying bone metabolism is required to more precisely characterize the impact of various PTH(1-84)/PTH(frag) ratios on skeletal remodeling in chronic renal failure. In this regard, estimates of the relative abundance of PTH(1-84) and other amino-terminally truncated PTH fragments did not prove useful in a recent report for identifying patients with adynamic renal osteodystrophy as documented by quantitative bone histomorphometry [79].

IMPLICATIONS OF RECENT DEVELOPMENTS FOR CLINICAL MANAGEMENT

While new developments intrigue investigators, they frustrate clinicians. It is increasingly apparent that values determined using first generation immunometric PTH assays are less reliable now than in the past as non-

invasive indicators of renal osteodystrophy, probably as a result of evolving therapeutic practices.

Current guidelines suggest that plasma PTH levels, when measured using first generation immunometric assays, should be kept between 150 and 300 pg/mL in patients with ESRD to maintain bone formation and turnover at relatively normal levels [1, 5, 66, 67]. The relatively higher normative values for plasma PTH in patients with ESRD compared to persons with normal renal and parathyroid gland function are thought to reflect skeletal resistance to the biological actions of PTH in renal failure. It is possible that N-terminally truncated, PTH-derived peptides, perhaps by signaling through a C-PTH receptor, contribute to this disturbance.

Nevertheless, plasma PTH levels that remain persistently higher than the recommended target range for patients with ESRD are typically associated with bone biopsy evidence of secondary hyperparathyroidism. In contrast, PTH levels less than 150 pg/mL, particularly those below 100 pg/mL, usually indicate adynamic renal osteodystrophy [1, 5, 6, 66]. Based upon published comparisons demonstrating approximately twofold differences between PTH values determined by first and second generation immunometric assays, a target PTH range of approximately 75 to 150 pg/mL as measured by second generation PTH assays would correspond to existing guidelines for the preferred concentration of PTH in plasma for patients with ESRD. Although marked disparities between results occur in some patients, the physiological importance of these findings is uncertain and the factors responsible for them are largely unknown. Despite commercial endorsements, there is little objective evidence to support the use of estimates of the concentration of PTH-derived peptide fragments in the diagnosis or management of renal osteodystrophy. Bone biopsy evidence of adynamic renal osteodystrophy is found in some patients whose plasma PTH levels exceed 500 pg/mL as measured by first generation immunometric PTH assays, but such individuals have often been treated with active vitamin D sterols and oral calcium supplements. Relatively low serum alkaline phosphatase levels may help to identify such cases [80].

Disturbances implicated as causes of adynamic bone in ESRD include diabetes, aggressive treatment of secondary hyperparathyroidism with vitamin D, the administration of large oral doses of calcium, and hypercalcemia. Further work is needed to determine whether these same variables influence the accumulation of N terminally-truncated PTH-derived peptides in serum or plasma and, thus, account for wide disparities between first and second generation immunometric PTH assays in some patients with ESRD. High blood ionized calcium concentrations affect the release and/or production of N terminally-truncated PTH-derived peptides, but the impact of these changes on bone metabolism remains uncertain. Caution is warranted, however, to avoid lowering plasma PTH levels excessively in patients with secondary hyperparathyroidism who are treated with active vitamin D sterols. Reductions in the dose of vitamin D are often necessary as plasma PTH levels decline and approach the therapeutic target range, to diminish the risk of hypercalcemia and to minimize the chances of developing adynamic bone [16].

New treatments such as calcimimetic agents offer the prospect of controlling secondary hyperparathyroidism without relying on treatment with vitamin D or with the use of much lower doses of vitamin D sterols. The wider availability of phosphate-binding agents that do not contain calcium, such as sevelamer, and the possible introduction of other calcium-free agents, such as lanthanum carbonate and iron-containing compounds, may limit exposure to very large oral doses of calcium. By circumventing interventions that can separately affect PTH secretion, plasma PTH measurements in the future may regain value as a useful indicator of bone turnover in patients with renal failure. Until these therapeutic approaches are established and characterized, it may be necessary to rely more frequently on bone histology to effectively manage renal osteodystrophy in patients with ESRD.

CONCLUSION

The recent characterization of additional modifiers of bone metabolism provides further insight into mechanisms to account for the development of certain types of renal bone disease. These developments are potentially important ones for the field of bone biology, and they offer expanded opportunities to better delineate the molecular mechanisms by which PTH and PTH-derived peptides modulate mineral metabolism and skeletal remodeling. Insufficient information is available, however, to extend these concepts into the clinical setting, and it is premature to apply these principles to the management of patients with renal osteodystrophy. Further work is required to adequately document the importance of PTH-derived peptides and signaling through a C-PTH receptor as key modulators of skeletal metabolism in chronic renal failure.

New second generation immunometric PTH assays measure full-length biologically active PTH(1-84) exclusively, and this feature distinguishes them from currently available first generation immunometric PTH assays. Results provided by first generation immunometric PTH assays will continue, however, to guide current management decisions because they are supported by abundant bone histology data. Whether second generation immunometric PTH assays provide more reliable information for the initial diagnosis and/or longitudinal follow-up of patients with renal bone disease remains to be determined.

Indeed, the interpretation of serum or plasma PTH values obtained using second generation immunometric PTH assays in patients with ESRD depends largely upon their correlation with first generation assays and only then by extrapolation to bone histology. Adequate supporting bone biopsy data have yet to be reported with second generation immunometric PTH assays to guide their use. If measurements are done using second generation immunometric PTH assays in patients with ESRD, guidelines for the interpretation of results relative to values expected using first generation assays should be provided to avoid untoward diagnostic or therapeutic outcomes.

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