PTH(1–84)/PTH(7–84): a balance of power

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RECENT YEARS HAVE WITNESSED remarkable progress in understanding parathyroid gland physiology in general and in further identifying various circulating forms of parathyroid hormone (PTH). The development of new immunometric assays for PTH that exclusively detect the full-length hormone composed of 84 amino acid residues, or PTH(1–84), only serves to underscore the relative abundance and potential importance of PTH fragments that are normally present in plasma. Several lines of evidence indicate that one or more fragments of PTH possess discrete biological activities and that some of these peptides may not simply represent inactive degradation products from the metabolism of PTH. Indeed, certain PTH fragments can alter signal transduction via classic pathways by affecting cytoplasmic adapter proteins that modify PTH receptor-mediated responses to classic PTH agonists, possibly by signaling separately through a novel PTH receptor. In this review, we endeavor to interpret these new findings and to integrate them into current knowledge about the biology of PTH, with special attention to their potential clinical and physiological significance.

BIOSYNTHESIS AND METABOLISM OF PTH

It is appropriate to begin any discussion of PTH with a brief description of the essential features of its synthesis and metabolism. PTH is an 84-amino acid peptide that is encoded in humans by a gene located on the short arm of chromosome 11 and in mice by a gene on chromosome 7 (83). The transcription product not only encodes the 84 amino acids of the mature peptide but also encodes both a “pre” signal sequence of 25 amino acids and a basic “pro” hexapeptide (Fig. 1). The 115-amino acid translation product is called preproparathyroid hormone, or pre-pro-PTH.

The NH₂-terminal 25 residues of pre-pro-PTH constitute the so-called the pre-, signal, or leader sequence. This signal sequence targets the peptide to the cellular secretory pathway, where it binds to a signal recognition particle that interacts with a docking protein on the endoplasmic reticulum membrane. As the nascent peptide is transferred to the intracisternal space of the endoplasmic reticulum, the single-chain pre-pro peptide is converted rapidly to PTH by cleavage of the NH₂-terminal signal sequence (36). The absence of the signal sequence, or mutations within this segment, can cause hypoparathyroidism (3, 47), thus emphasizing the importance of the signal sequence for proper PTH processing and secretion.

The remaining hexapeptide pro sequence is then hydrolyzed by trypsin-like endopeptidases in the Golgi apparatus. The resulting mature 84-amino acid hormone is subsequently packaged into secretory granules and transported to the cell membrane. Little pro-PTH is stored within the gland. Pro-PTH itself has negligible biological activity and is not detectable in the general circulation (63).

Two forms of secretory granules have been identified in parathyroid cells. One contains the full-length 84-amino acid PTH. The other type of granule contains both PTH and the proteases cathepsins B and H (40). Cathepsin B cleaves PTH to form PTH(37–84) and a mixture of NH₂-terminal hormone fragments (53). These and perhaps other cysteine proteases thus generate a number of peptide fragments that have been thought traditionally to be biologically inactive. It is now apparent, however, that the metabolism of PTH is regulated, most notably by variations in blood ionized calcium concentration.

PTH secretion is controlled by the extracellular calcium-sensing receptor (CaSR), a G protein-coupled receptor on the surface of parathyroid cells. Unlike most secretory cells, how-
never, low extracellular calcium promotes, rather than inhibits, PTH(1–84) release from parathyroid cells. Conversely, CaSR activation by elevated extracellular calcium causes the release of intracellular calcium, and in turn inhibits secretion of PTH(1–84) (see Fig. 2). Inhibition of PTH(1–84) secretion is accompanied by enhanced proteolysis of the NH2-terminus of PTH, with the attendant secretion of PTH(7–84) and other NH2-truncated PTH peptide fragments (see Fig. 2).

Again, in response even to modest reductions of serum calcium, relatively more PTH with an intact NH2 terminus, as detected by various immunoassay methods, is present in the circulation compared with NH2 terminally truncated peptides that are detected by assays that target epitopes located in the mid- or COOH-terminal portions of PTH. By interacting with the type I PTH receptor, or PTH1R, such NH2-terminally intact peptides act in kidney and bone to restore blood ionized calcium levels to normal. In contrast, relatively more COOH-terminal PTH fragments are found when blood ionized calcium concentrations are elevated (25, 26). Whether this change simply reflects an alteration in the metabolism of PTH(1–84) under conditions of calcium surfeit (16, 56) or whether NH2 terminally intact peptides actually participate directly in the regulation of blood ionized calcium concentrations is uncertain based on recent experimental observations. Although the circulating concentration of free calcium dramatically affects PTH secretion and the secreted forms of PTH, the peripheral metabolism of PTH is unaffected by changes in calcium over an extensive range (8).

At lowered extracellular calcium concentrations, immunoreactivity for cathepsins B and H and for PTH is reduced in parathyroid cells without a change in lysosomal proteinase immunoreactivity (40). Such findings suggest that calcium, presumably acting through the CaSR, regulates parathyroid cathepsin activity specifically and thereby modulates the relative abundance of the various molecular forms of PTH that are secreted. In contrast, variations in calcium concentration do not influence the enzymatic cleavages involved in the processing of pre-pro- or pro-PTH (41). Cathepsins B and H are also found in hepatic Kupffer cells (82), another primary site of PTH degradation, but it is not known whether their activities are modulated by changes in extracellular calcium.

In the face of high circulating levels of PTH associated with experimental renal failure, renal tubular proteinase activities are also suppressed (66). This may indicate an additional functional feedback mechanism regulated by proteolytic degradation of PTH by cysteine proteinases that determines the abundance of biologically active circulating levels of PTH.

Overall, these experimental findings suggest that changes in extracellular calcium concentration not only regulate the secretion of PTH(1–84) by the parathyroid glands but also...
modulate the relative abundance of various PTH-derived peptides that are released into the circulation. Variations in blood ionized calcium may also modify the peripheral metabolism of PTH(1–84). The potential physiological importance of these observations in the regulation of calcium homeostasis and bone remodeling is currently the subject of considerable scrutiny.

**MEASUREMENT OF CIRCULATING FORMS OF PTH**

PTH(1–84) and a variety of peptide fragments derived from it are present simultaneously in peripheral blood. Indeed, the concentration of peptide fragments is several-fold greater than the concentration of the intact hormone. Some of these fragments arise from the metabolism of PTH(1–84) in various tissues, most notably liver, whereas others are released from the parathyroid glands directly, probably reflecting the intracellular degradation of PTH(1–84) within parathyroid cells. The identification and quantification of the abundance and heterogeneity of PTH-derived peptides in plasma have proved to be a major obstacle to the development of reliable PTH assays because it is now appreciated that some of these peptide fragments unknowingly interacted with the clinical assays used to determine PTH levels.

Early assays for PTH, such as those developed during the 1960s and others that were used extensively into the late 1980s, relied primarily on radioimmunoassay (RIA) methods (4, 5). Isotopically labeled peptides, usually highly purified full-length PTH(1–84) or synthetic PTH fragments, were used in competitive displacement assays with antibodies that recognized particular epitopes within the PTH molecule. Such antibodies most often, however, targeted epitopes located in the midportion or COOH-terminal region of PTH(1–84) (Fig. 1). Because plasma normally contains an abundance of PTH-derived peptides in addition to PTH(1–84), most RIAs for PTH cross-reacted with and thus detected a variety of peptide fragments lacking one or more amino acid residues from their NH2-terminal end.

NH2 terminally truncated fragments of PTH(1–84), also known as COOH-terminal PTH fragments, are generally incapable of binding effectively to the PTH1R and initiating signal transduction. Accordingly, they are inactive biologically with respect to the classic actions of PTH. Because such peptides were readily detected by single-antibody RIAs for PTH, they interfered quite markedly with efforts to measure the concentration of PTH(1–84) in samples of serum or plasma. The results provided by most PTH RIAs were thus quite variable and not highly reproducible. This was most problematic among patients with chronic kidney disease, who retain excess amounts of PTH-derived peptides in plasma because both the clearance of these peptides from plasma and their subsequent degradation in the kidney are diminished when renal function is impaired. Measurements using RIAs for PTH failed to provide reliable assessments of parathyroid gland function or to predict accurately the skeletal manifestations of hyperparathyroidism among patients undergoing dialysis regularly (2, 64).

Certain RIAs for PTH employed antibodies that targeted epitopes within the NH2-terminal portion of PTH(1–84). Such assays were designated as NH2-terminal PTH assays. Some were reported to provide better diagnostic information than midregion or COOH-terminal PTH RIAs for the overall assessment of renal bone disease and for distinguishing between patients with either high-turnover or low-turnover skeletal lesions as documented by bone histology among those undergoing dialysis (2). The greater predictive value of NH2-terminal PTH RIAs in the assessment of renal osteodystrophy was most likely due to the fact that the assays interacted predominantly with the portion of PTH(1–84) that is responsible for binding to the PTH1R and for mediating its biological actions in target tissues.

The introduction of dual-antibody, immunometric PTH assays circumvented many of the shortcomings of single-antibody RIAs (1, 62). Immunometric PTH assays employ two antibodies that recognize discrete epitopes located in different portions of PTH(1–84). One antibody interacts with an epitope located within the NH2-terminal region of PTH, whereas the other interacts with an epitope located in the midregion or COOH-terminal portion of the molecule (Fig. 2). Because both antibodies are required for detection in the assay procedure, only relatively long peptides containing both targeted epitopes are recognized by double-antibody immunometric methods (33). Small peptides lacking one or both epitopes are not detected.

When first introduced and for many years thereafter, immunometric PTH assays were thought exclusively to detect full-length, biologically active PTH(1–84). Recent work indicates, however, that most first-generation immunometric PTH assays detect one or more peptides distinct from PTH(1–84) (31, 44, 51). Some of these peptide fragments segregate into the same fraction as synthetic PTH(7–84) on high-performance liquid chromatography (9, 10, 51). Nevertheless, it is likely that first-generation immunometric PTH assays also cross-react with a variety of other NH2 terminally truncated fragments of PTH(1–84) because the epitopes targeted by the labeling antibodies used in these assays are located some distance from the NH2 terminus. Thus by varying degrees first-generation immunometric PTH assays also overestimate the true concentration of PTH(1–84) in serum or plasma.

Second-generation immunometric PTH assays have been developed that appear solely to detect PTH(1–84). These assays do not detect synthetic human PTH(7–84) and would not be expected to cross-react with other large NH2 terminally truncated peptides such as synthetic PTH(3–34) or PTH(19–84), although data that address this issue directly have yet to be reported (44). The labeling antibodies used in second-generation immunometric PTH assays are directed toward epitopes located within the most NH2-terminal portion of the molecule (44). It is this feature that confers their specificity for PTH(1–84) compared with first-generation assays. Synthetic PTH peptides lacking one or more amino acid residues such as PTH(2–34) and PTH(3–34) do not compete effectively for binding by the labeling antibodies utilized in second-generation immunometric PTH assays (44, 65).

It should be noted, however, that the second antibody used in most second-generation PTH assays targets an epitope within the region of the peptide hormone composed of amino acid residues 44–65. It is likely, therefore, that second-generation PTH assays are also capable of detecting fragments of PTH(1–84) that are intact fully at their NH2 terminus but that are missing one or more amino acid residues from their COOH-terminal end. Additional work is required to address this issue and its potential pathophysiological significance.
When PTH levels are measured in the same plasma sample using both first-generation and second-generation immunometric assays, the results show a high degree of correlation not only in persons with normal renal function but also in those with chronic kidney disease (31, 44, 69). The values obtained using second-generation assays are ~40–50% lower than those provided by first-generation assays across a wide range of plasma PTH concentrations (31, 44, 59, 69). Such findings indicate that PTH(1–84) accounts for slightly more than half of the immunoreactivity detected by first-generation immunometric PTH assays, whereas various NH2 terminally truncated peptide fragments of PTH(1–84) probably account for the remaining immunoreactivity detected by these methods.

Interestingly, the relative abundance of NH2 terminally truncated PTH fragments, as estimated by the disparity between the results obtained by first- and second-generation PTH assays, does not differ substantially between volunteer subjects with normal kidney function and patients with markedly impaired renal function who require treatment with dialysis. Such findings are noteworthy because reductions in glomerular filtration and in protein catabolism by renal tubular epithelial cells are thought to account primarily for the accumulation of excess amounts of PTH-derived peptides in the circulation of patients with chronic kidney disease. Although short-term variations in serum calcium concentration have been shown to influence the relative abundance of PTH(1–84) compared with estimated amounts of NH2 terminally truncated PTH fragments (12, 16), this relationship does not appear to be affected materially by variations in renal function under steady-state conditions.

These findings suggest a greater importance than heretofore appreciated for the generation and release of NH2 terminally truncated PTH fragments by the parathyroid glands at elevated levels of extracellular calcium (48).

**EFFECTS OF PTH ON RECEPTOR ACTIVATION AND RECEPTOR TURNOVER**

PTH(1–84) and its shorter synthetic analog, PTH(1–34), activate a number of signal transduction pathways both classic, i.e., adenylyl cyclase and phospholipase C (Fig. 3), and non-classic, e.g., Erk, phospholipase A2, and phospholipase D. The structural determinants responsible for stimulation of adenylyl cyclase and of phospholipase C have been defined and reviewed comprehensively (60). Briefly, PTH1R signaling through adenylyl cyclase requires the first two amino acid residues of PTH. Elimination of Ser1 in human PTH or Ala1 in bovine or porcine PTH reduces maximal cAMP formation. Combined removal of Ser/Ala1 and Val2 virtually eliminates adenylyl cyclase activation. Peptides lacking the first three to six amino acids are inhibitors of PTH action (23, 42).

Proteolysis of the NH2 terminus of PTH has long been considered to render the remaining fragment inert and serves as a means for reducing the amount of active, full-length PTH (37). A remarkable convergence of information obtained using new immunometric assays for PTH as described above, together with recent work on PTH receptors, suggests that certain NH2 terminally truncated fragments of PTH(1–84) including PTH(7–84) offset the classic biological actions of PTH that are
mediated through the PTH1R and in some instances activate nonclassic PTH signaling pathways (72, 73). These effects occur by a combination of promoting receptor internalization and downregulation without antecedent or concurrent receptor activation of adenyl cyclase or phospholipase C. Moreover, these actions are not due to the competitive inhibition of binding of NH2-terminal PTH fragments or of PTH(1–84) to the PTH1R but represent a discrete biological action of NH2 terminally truncated PTH peptides on PTH1R endocytosis. Such effects occur in a strikingly cell-specific manner, as described below. Although such inhibitory actions are likely to be important physiologically, their biological role is not yet understood fully. The principal and known actions of PTH(1–84) and of PTH(7–84) as a prototype of NH2-truncated PTH peptide fragments are summarized in Table 1.

Recent studies show that PTH peptides that are incapable of activating classic signaling pathways through the PTH1R efficiently internalize the receptor (70, 71, 73). PTH peptides lacking the first three to nine amino acids caused PTH1R endocytosis, with the magnitude and rapidity of internalization being inversely proportional to the length of the fragment and the degree to which it activated adenyl cyclase. Fragments lacking the first 10 amino acids [PTH(10–34)] had no effect on PTH1R internalization or on PTH1R-dependent signaling. These findings were the first to demonstrate that PTH1R activation and inactivation could be dissociated. While inherently interesting for what it disclosed about the biology of G protein-coupled receptors, the results had little physiological relevance, insofar as the effects were achieved with short, synthetic peptide fragments that are not known to be produced by the metabolic degradation of full-length PTH and are not thought to be present in the peripheral circulation.

It became important to explore this issue further because emerging evidence indicates that large NH2 terminally truncated PTH fragments such as PTH(7–84) are present normally in peripheral blood and accumulate appreciably in end-stage kidney disease (15, 51). We sought, therefore, to determine whether larger biologically relevant forms of NH2 terminally truncated PTH exerted similar effects on PTH1R internalization as demonstrated previously with shorter synthetic peptides. Both PTH(1–84) and PTH(7–84) behaved similarly in this regard and in a manner entirely comparable to the results obtained with their respective shorter synthetic PTH analogs. The fact that PTH(7–84) internalized the PTH1R without prior receptor activation suggests additional novel explanations for the downregulation of PTH1R expression in uremia; for the tissue resistance to PTH that characterizes chronic renal failure; and for the dissociation of phosphorus and calcium homeostasis in chronic kidney disease.

The precise origins of PTH resistance in uremia are incompletely understood. Two principal theories have been advanced to explain the phenomenon, especially as it relates to the pathogenesis of adynamic renal osteodystrophy, which some investigators have attributed to the excessive accumulation in plasma of NH2 terminally truncated PTH fragments, one of which is PTH(7–84). When administered concurrently with PTH(1–84) at a molar ratio of 1:1, PTH(7–84) antagonized the calcemic and phosphaturic actions of PTH(1–84) in parathyroidectomized rats (69). Such findings led Slatopolsky et al. (69) to suggest that PTH(7–84) acts as a competitive inhibitor of PTH(1–84) at the level of the PTH1R. This explanation is unlikely, however, because downregulation of the PTH1R has been described in the same pathological setting (76, 78). Receptor downregulation would be inconsistent with a competitive mechanism of action by PTH(7–84), which would be expected to increase rather than decrease PTH1R expression by offsetting PTH(1–84)-mediated receptor endocytosis and degradation.

An alternative and more likely explanation to account for skeletal and renal resistance to PTH in chronic renal failure comes from the work of Bringhurst and associates (10, 19–21). They provided compelling evidence for a novel, COOH-terminal PTH receptor (C-PTHR) with high-affinity binding to COOH-terminal PTH fragments. Signaling through a putative C-PTHR, which has yet to be cloned, might conceivably mediate or otherwise account for the tissue resistance to the classic biological actions of PTH that proceed through the PTH1R, although just how such a receptor might be involved is not currently known.

Based on observations that 1) the plasma levels of PTH(7–84) and other NH2 terminally truncated PTH fragments levels are conspicuously elevated in end-stage renal failure, 2) the PTH1R is downregulated in this circumstance, and 3) PTH(7–84) internalizes the PTH1R in selected target cells in kidney and bone, it is possible that PTH(7–84) contributes importantly to the PTH resistance of renal failure by internalizing and downregulating the PTH1R (29). According to this view, the PTH1R is sequestered and downregulated in cells that lack sufficient amounts of ezrin-binding protein of 50 kDa (EBP50)/Na+/H+ exchanger regulatory factor 1 (NHERF1), as demonstrated recently in cells of the distal nephron. Thus, in distal tubular epithelial cells that do not constitutively express EBP50/NHERF1, the PTH1R is internalized by PTH(7–84), and this change is associated with reductions in PTH-mediated epithelial calcium transport. In contrast, downregulation of the PTH1R does not occur in epithelial cells of the proximal nephron that express EBP50/NHERF1 after exposure to PTH(7–84). Here, the inhibitory effect of PTH on epithelial phosphorus transport should be unaffected.

The predicted integrated consequences of these biological effects would be resistance to the hypercalcemic action of PTH but preservation of its phosphaturic effect. This constellation of actions, where the effects of PTH on proximal phosphate excretion and distal calcium conservation are dissociated, was recognized by Llach and associates (52) many years ago in clinical studies of patients with mild to moderate renal failure. They considered the possibility that elevated immunoreactive PTH (iPTH) levels in chronic renal failure may not reflect a biologically active hormone. It is now evident that the presence
of PTH(7–84) and other NH₂ terminally truncated PTH fragments are the likely explanations for their prescient analysis.

By contrast, some investigators have suggested that the resistance of bone to the remodeling effects of PTH can be ascribed to uremia per se (28). In studies of rats with different degrees of experimentally induced renal failure, Bover et al. (6) noted that animals with moderate reductions in renal function that were maintained on a low-phosphorus diet had PTH levels consistently greater at any given concentration of serum calcium compared with rats with normal renal function. Moreover, the calcemic response to intravenous infusions of PTH was diminished in animals with diminished kidney function. Such findings suggest that the capacity of PTH to raise serum calcium levels may be impaired directly as a consequence of uremia. It should be noted, however, that persistently elevated PTH levels would be expected to desensitize the PTH1R and attenuate all of the actions of PTH.

Although concurrent measurements of PTH(1–84) and PTH(7–84) have been suggested to provide a better biochemical indicator of bone turnover among patients with renal osteodystrophy due to advanced kidney failure (59), further work is needed to define more precisely the mechanisms responsible for PTH resistance in uremia, and the role, if any, of PTH(7–84) and other NH₂ terminally truncated PTH peptides in this disturbance.

**RELATIONSHIP BETWEEN PTH LEVELS AND BONE TURNOVER IN RENAL FAILURE IN HUMANS**

Measurements of PTH levels in serum or plasma are used widely for the initial diagnosis of renal bone disease; to monitor its evolution over time; and to assess responses to treatment. In practical terms, the results are employed as a noninvasive biochemical surrogate for bone biopsy and quantitative bone histomorphometry due to realistic constraints imposed by the invasive nature of the bone biopsy procedure and the limited availability of the specialized laboratory resources needed to process bone biopsy specimens and to provide suitable histological analyses. Nevertheless, the value of any particular PTH assay for assessing patients with renal osteodystrophy largely depends on the extent to which biochemical results have been validated by bone histology in published clinical studies.

Substantial bone histological data, with concurrent measurements of plasma PTH levels, have been reported using first-generation immunometric PTH assays among patients undergoing long-term dialysis. The results provide the basis for the recommendation that plasma PTH levels be maintained within the range of 150–300 pg/ml to minimize the histological abnormalities of renal bone disease and to maintain relatively normal rates of bone formation and turnover in such patients (24). In contrast, values >300–400 pg/ml are usually associated with histological evidence of secondary hyperparathyroidism, whereas levels <150 pg/ml, and particularly values <100 pg/ml, are characteristic of patients with biopsy-proven adynamic renal osteodystrophy.

Much less is known about the relationship between plasma PTH levels and bone histology among patients with chronic kidney disease who do not require treatment with dialysis. Few studies have been done. Available results suggest, however, that histological evidence of secondary hyperparathyroidism is common when plasma PTH levels exceed 80–100 pg/ml as measured by first-generation immunometric assays among patients with creatinine clearances in the range of 15–50 ml/min. It thus appears that the skeletal manifestations of secondary hyperparathyroidism occur at much lower plasma PTH levels in those patients with some residual renal function compared with patients with little or no remaining kidney function and who require treatment with dialysis.

Only limited information is available about the usefulness of measurements of plasma PTH levels, as determined by second-generation immunometric assays, as predictors of bone histology among patients with chronic kidney disease. One report suggests that values in the range of 80–160 pg/ml are associated with relatively normal bone histology and normal rates of bone formation among patients undergoing dialysis regularly (50). Results above this range generally corresponded with histological evidence of hyperparathyroidism, whereas values below this range were associated with bone biopsy evidence of adynamic renal osteodystrophy. Confirmation of these preliminary findings is required, but the results are not unexpected based on the previously documented high degree of correlation between values from first- and second-generation PTH assays.

Despite improvements in the reliability of measurements provided by first- and second-generation PTH assays, diagnostic uncertainties persist when these methods are used as a noninvasive biochemical predictor of bone histology among patients with renal osteodystrophy. Certain of these difficulties are related to the fact that the correlations between plasma PTH levels, as determined by first-generation immunometric assays, and bone histology were established most robustly in studies undertaken during the 1980s and early 1990s, the results of which were reported more than 10 years ago. Such findings may not reflect this relationship accurately among patients now receiving long-term dialysis or with results of second-generation immunometric assays.

The demographics of the end-stage renal disease population have changed substantially during the past 10–15 years. Nearly one-half of patients undergoing dialysis currently in the United States are diabetic, and more than one-half of them are over 65 yr of age. Diabetes and aging are known to affect adversely bone metabolism and skeletal remodeling, and each can contribute to the development of adynamic renal osteodystrophy by mechanisms distinct from those mediated by alterations in parathyroid gland function. The high proportion of diabetic and elderly patients in the current dialysis population thus undermines confidence about the predictive value of biochemical guidelines established originally in a substantially different patient population as a noninvasive index of bone histology in contemporary clinical practice.

Against this historical background, it has been suggested that the accumulation of NH₂ terminally truncated fragments of PTH in some patients undergoing dialysis accounts for reductions in bone formation and turnover and for the development of adynamic renal osteodystrophy (59). The introduction of second-generation immunometric PTH assays that exclusively detect PTH(1–84) provides a tool to explore this possibility. Because first-generation immunometric assays detect both PTH(1–84) and various peptide fragments as discussed previously, the difference in plasma PTH levels as determined by first- and second-generation PTH assays has been used to estimate the plasma concentration of NH₂ terminally truncated
fragments. Such estimates are not direct measurements of the concentration of PTH(7–84) as is often stated incorrectly. Rather, the values most probably reflect the plasma level of a variety of NH2 terminally-truncated fragments, one of which is PTH(7–84).

Monier-Faugere and colleagues (59) presented the initial evidence to suggest that the disproportionate accumulation of NH2 terminally truncated PTH fragments relative to PTH(1–84) served to identify patients with adynamic renal osteodystrophy among those regularly undergoing dialysis. Additional information to support this contention has not been forthcoming, however, and other investigators have failed to confirm these findings (13). Given the paucity of data from clinical studies using bone histology to validate such biochemical findings, it is thus premature to ascribe a fundamental role for NH2 terminally truncated peptides in general, and for PTH(7–84) in particular, in the pathogenesis of adynamic renal osteodystrophy. Additional information about vitamin D nutritional status, recent treatment with vitamin D sterols, and the ambient serum calcium concentration at the time blood is obtained for PTH measurements may also be required. More definitive work is thus needed to determine whether one or more NH2 terminally truncated fragments of PTH modulate calcium metabolism or influence skeletal remodeling in humans.

Despite the shortcomings of currently available clinical data, several observations have heightened interest in the potential importance of peptide fragments of PTH that have traditionally been considered to be inactive biologically. Studies in various experimental models indicate that synthetic PTH(7–84) and other NH2 terminally truncated PTH fragments can counteract the classic biological actions of PTH(1–84) and PTH(1–34) in regulating serum calcium levels, actions that are mediated by signal transduction through the PTH1R. Both PTH(7–84) and other synthetic NH2 terminally truncated peptides have been shown to modify calcium efflux from neonatal mouse calvariae and to influence osteoclast differentiation in vitro (20, 21, 61). Osteocytes derived from genetically modified mice lacking the PTH1R exhibit high-affinity binding for PTH(19–84) that is presumably mediated by a putative C-PTHR as described previously in osteoblast-like cells (19, 21, 22). Moreover, binding of the COOH-terminal portion of PTH to the putative C-PTHR in osteocyte-like cells promotes apoptosis (7, 20). It is thus plausible that peptide fragments of PTH(1–84) serve a physiological function by interacting with a discrete C-PTHR to alter bone cell metabolism and to offset or modify signal-transduction via the PTH1R (59, 69). Such a mechanism could account for the tissue resistance to PTH that characterizes patients with advanced renal failure and contribute to alterations in bone metabolism among those with chronic kidney disease.

In renal tubular epithelial cells, the effect of different molecular species of PTH on signal transduction through the PTH1R and on the level of PTH1R expression appears to be modulated in a cell-specific manner. Scaffolding proteins such as NHERF may be involved. NHERF1 is expressed in osteoclasts (35), but it is not yet clear whether NHERF1s are expressed in osteoblasts or in osteocytes. NHERF1 might conceivably prevent or promote vascular calcification in renal disease. If PTH(1–84) promotes skeletal mineral accumulation while concurrently limiting vascular calcification, the accumulation of PTH fragments may contribute to the calcific vascularopathy of end-stage renal failure (67). Expression of NHERF1 prevents PTH(7–84)-dependent PTH1R internalization. Thus, if NHERF1 is expression is downregulated in renal failure, PTH(7–84) would contribute to the blunted PTH sensitivity of uremia via enhanced cell-surface PTH1R endocytosis (80). Conversely, if NHERF1 expression is upregulated in this setting, ectopic calcification may be blunted. Nevertheless, variations in the level of expression of NHERF1 in distinct populations of bone cells represent a potentially important mechanism to account for changes in PTH1R-mediated signal transduction that are mediated by NH2-truncated PTH fragments, as described earlier.

INVOLVEMENT OF PTH IN EXPERIMENTAL MODELS OF RENAL FAILURE

Definitive elucidation of the role of PTH peptides, receptor downregulation, and the role of NHERF in shielding the PTH1R from the internalizing effect of PTH(7–84) in renal failure will require further investigation. A fairly substantial literature, however, exists from extant studies of experimental models of renal failure, generally in rats, that permits some speculation on possible NHERF involvement in mineral ion homeostasis in this setting. The overall goal of such studies has been to uncover the interactions among serum PTH, calcium, phosphorous, and the magnitude of loss of renal function. Animals have generally been subjected to subtotal (5/6) nephrectomy, either with or without parathyroidectomy. Additional interventions have included dietary manipulations, generally of phosphate but also of protein (68), the administration of PTH, most often continuously and generally PTH(1–34) (75), treatment with vitamin D sterols (74), and the induction of metabolic acidosis (43).

In experimental animals, as in humans, progressive renal failure is associated with reductions in the fractional reabsorption of phosphorus within the nephron. Advanced chronic kidney disease, CKD3–5 (60a), is almost invariably accompanied by secondary hyperparathyroidism in individuals who are untreated. The hallmarks of secondary hyperparathyroidism due to chronic kidney disease include reductions in renal calcitriol production with diminished serum levels of 1,25(OH)2D3, decreases in intestinal calcium absorption that result in hypocalciuria, a tendency toward mild or overt hypocalcemia, and, at later stages, hyperphosphatemia. As described here, the question to be addressed is whether certain of these disturbances are attributable, either wholly or in part, to the biological actions of PTH peptides with an intact NH2 terminus, such as PTH(1–84) and PTH(1–34), that interact with the PTH1R or possibly to one or more NH2 terminally truncated PTH fragments that accumulate in renal failure and that potentially interact with a discrete COOH-terminal PTH receptor (9, 11). Resolving this problem is important both for better understanding bone metabolism in chronic kidney disease and for refining the diagnosis and proper clinical management of renal osteodystrophy. Unfortunately, a complete answer to the question is not yet available.

Insights have been gleaned, however, from studies of animals with experimental renal failure, and the results provide suggestions for more definitive investigation. The PTH1R is downregulated in the kidney and in the epiphyseal growth plate.
cartilage of rats with surgically induced renal failure (77, 79). PTH(7–84), given either alone (49) or in combination with PTH and other PTH fragments that accumulate in renal failure (61), decreases serum calcium concentrations in parathyroidectomized rats and antagonizes the effect of PTH(1–84) on bone. It would be attractive to attribute this effect exclusively to the presence of PTH(7–84), which internalizes and down-regulates the PTH1R in cell culture models of bone and kidney (72). Downregulation of the PTH1R in vivo cannot, however, account for an effect of PTH(7–84) or any other PTH peptides because parathyroidectomy fails to prevent receptor downregulation (79). Nonetheless, a circulating factor is strongly implicated in PTH1R downregulation because ultrafiltrates prepared from the serum of uremic patients cause downregulation of the PTH1R in UMR 106 osteoblast-like cells (18). The factor involved is not PTH(1–34), because the addition of this particular peptide to uremic serum or the persistent exposure of UMR-106 cells to PTH(1–34) fails to diminish cAMP formation. Possible involvement of PTH cannot be entirely excluded because it is now recognized that PTH is synthesized and in a regulated manner by the thymus in mice (34) and intrathymic adenomas in humans (55).

The effects of magnesium on PTH secretion mirror those of calcium but with a potency that is several-fold lower. Mild hypomagnesemia stimulates PTH secretion, whereas hypermagnesemia suppresses PTH secretion. In contrast, severe magnesium depletion impairs PTH secretion and the phosphaturic action of PTH (32, 58), whereas markedly elevated magnesium levels also suppress PTH secretion. More complicated interactions occur, however, between magnesium and calcium that affect PTH secretion. Low serum magnesium levels impair PTH secretion that is normally enhanced by reduced serum calcium concentrations (81). Nevertheless, changes in serum magnesium or serum calcium concentrations are alone insufficient to explain either the reduced receptor abundance or receptor-mediated responses in either circumstance (79).

It should be noted that downregulation of PTH1Rs is tissue specific because it does not occur in either the liver or heart (79). This may implicate tissue-specific PTH1R promoters with distinct regulatory capability (46, 57). Systemic acidosis directly downregulates the PTH1R, at least as assessed by mRNA expression in isolated rat proximal tubules (45).

Treatment with the calcimimetic agent cinacalcet (Sensipar), an allosteric activator of the CaSR, reduces PTH secretion both in humans and in rats, and it attenuates parathyroid hyperplasia in subtotally nephrectomized rats (14). No change in serum phosphate is observed. These findings thus suggest that the CaSR rather than serum phosphate is the dominant factor regulating parathyroid proliferation. Serum PTH in these studies was measured with a rat PTH(1–34) immunometric assay (Immutopics, San Clemente, CA) that does not distinguish between biologically active PTH(1–34) and other circulating NH2-truncated PTH fragments such as PTH(7–34). Indeed, to the best of our knowledge, PTH(7–34) or PTH(7–84) levels have not yet been estimated in any experimental animal model. However, given the observation that hypercalcemia is attended by increased secretion of NH2-truncated PTH fragments (26, 37–39), it seems likely that, as in humans, there will be both increased secretion and accumulation of NH2-truncated PTH fragments in this setting. What remains to be determined is whether in experimental renal failure PTH(7–84) directly contributes to changes in PTH receptor expression or to the vascular calcification and bone demineralization that are pathological hallmarks of chronic kidney disease.

SUMMARY AND CONCLUSIONS

Laboratory studies provide intriguing clues for potential biological actions of NH2 terminally truncated PTH peptides. Although biological effects of PTH-inhibitory fragments can be demonstrated experimentally, it is as yet unclear whether they have diagnostic or clinical relevance. The preponderance of data at the present time fails to provide a convincing case that any PTH assay is superior to any other in predicting adynamic bone disease or serum concentrations of bone markers. Although much has been accomplished with respect to delineating the presence of significant quantities of PTH fragments, unequivocal structural identification of the non-PTH(1–84) peptide that accumulates in renal failure would facilitate determining a potential role in the etiology of chronic kidney disease and might serve as a surrogate indicator for diagnosis and treatment.

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REFERENCES

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