Parathyroid Hormone: New Assays, New Receptors

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Accurate measurements of parathyroid hormone (PTH) in plasma are necessary for the assessment, monitoring, and therapy of disorders of bone and mineral metabolism including renal osteodystrophy. Assays for PTH have evolved to provide 2-site immunometric assays that are highly specific for the intact 84 amino-acid peptide, PTH (1-84). With the advent of such assays, it has been shown that the prior generation of assays, thought to measure intact PTH, in fact, also detected a PTH peptide that was truncated at the N-terminus and that appeared to be similar to PTH (7-84). There has been renewed interest in such circulating PTH fragments in view of the demonstration that PTH (7-84) (and other PTH peptides) might have biologic effects. These effects include an action to oppose the calcemic effect of PTH in vivo and to inhibit bone resorption and osteoclast generation in vitro. These effects appear to be mediated by actions of a receptor for PTH peptides with specificity for the C-terminal region of PTH and distinct from the PTH receptor known to be responsible for all of the classic actions of PTH. Although the C-PTH receptor has not yet been cloned, the observations have opened a new field of research in parathyroid physiology. Clinical applications of the assay of such PTH fragments in relation to the amount of circulating PTH (1-84) concentrations are being sought actively as the new PTH assay methodology is applied to the clinical arena and as the biology of the C-PTH receptor and C-terminal PTH fragments are investigated.

BECAUSE PARATHYROID hormone (PTH) plays a major role in the regulation of bone and mineral metabolism, an accurate assessment of the activity of the parathyroid glands is necessary in a variety of clinical circumstances, including chronic kidney disease. In kidney disease, as kidney function declines, alterations in PTH activity contribute to the complex disorders of bone, which is referred to as *renal osteodystrophy*. Increased levels of PTH as a result of secondary hyperparathyroidism are associated with increased bone turnover, and in severe cases, osteitis fibrosa. In some patients with renal failure, however, bone turnover is abnormally low and is termed *adynamic bone* and is associated with relatively low levels of PTH. Some other patients show features of hyperparathyroidism together with evidence of defective mineralization and is termed *mixed renal osteodystrophy*. Because of this spectrum of skeletal abnormalities in chronic renal failure and the important role of PTH, accurate measurements of PTH levels are essential for the diagnosis, monitoring, and treatment of these skeletal disorders.

ASSAY OF PTH

The first radioimmunoassay for PTH was described in 1963 by Berson et al.[1] This demonstration sparked the development of several such assays, most of which used polyclonal antisera generated against intact PTH, an 84-amino acid peptide, which was purified from parathyroid glands. The region of the PTH molecule recognized by these assays varied considerably, but many were located toward the middle, or C-terminus, of the PTH sequence, giving rise to assays that were termed *midregion* or *C-terminal PTH* assays.[2] By using such assays, the presence in the circulation of fragments of PTH, in addition to intact PTH 1-84 soon was shown,[3] and it was discovered that these PTH fragments were derived from direct secretion from the parathyroid glands,[4-7] as well as being generated by peripheral metabolism of PTH 1-84 in organs such as the liver and kidney.[8-13] Although these initial radioimmunoassays provided valuable clinical diagnostic ability, and allowed for characterization of the secretion and metabolism of PTH and the pathophysiology of renal osteodystrophy, there was considerable heterogeneity of results obtained by different investigators.[2] This was especially problematic in the setting of renal failure because it was discovered that fragments of PTH from the middle and C-terminal regions of the PTH molecule depended on glomerular filtration for elimination from the body, and therefore accumulated to marked degrees in the setting of renal failure.[13] Thus, in advanced chronic renal failure, intact PTH (1-84) may represent only approximately 5% of total C-terminal or midregion immunoreactivity.[14]

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Efforts then were directed toward the development of radioimmunoassays directed toward the N-terminus of the PTH molecule, the region of PTH that was associated with the known biologic actions of PTH, which had been shown to reside within the first 34 amino acids of the peptide. Although such assays had theoretic benefits, in practice, relatively poor sensitivity severely limited their clinical use.[2] The shortcomings of these radioimmunoassay techniques resulted in the development of 2-site, sandwich-type assays, which offered the possibility of selecting PTH (1-84) from the complex mixture of intact hormone and fragments that existed in the circulation.[15-17] This strategy used 2 different affinity-purified antibodies directed toward different regions of the PTH molecule. The usual format was to use one antibody directed toward the C-terminal region of PTH, immobilized to a solid support, to capture PTH from plasma. Nonbinding PTH fragments and other proteins would then be washed away, and a second antibody, usually directed toward an amino terminal epitope of PTH, was used as a detection antibody when either radiolabeled, biotinylated, or enzyme-linked. This assay strategy (2-site immunometric assay) allowed high sensitivity and increased specificity compared with the prior radioimmunoassays, and thus enabled the detection of intact PTH and excluded PTH peptides that did not have antigenic determinants in both the N- and C-terminal regions of the PTH molecule. Thus, this assay system was termed an *intact PTH* immunoradiometric assay if radioactive tracer was used, or an immunochemiluminescent assay if chemiluminescence was used as a detection system. This assay technique also eliminated nonspecific serum effects, had excellent performance characteristics, and was adapted easily to more automated procedures suitable for broad clinical use. A variety of such 2-site assays for PTH have been in use all over the world for the past decade.

However, in 1993, during studies to evaluate the effect of calcium on circulating PTH levels, Brossard et al,[18] using high-performance liquid chromatography (HPLC) to separate the molecular forms of circulating PTH, noted that the 2-site immunometric assay, described earlier, which was thought to measure intact PTH 1-84, also appeared to react with an additional molecular form of PTH that eluted on HPLC just before the intact PTH peak. They termed this PTH molecular form *non–(1-84) PTH*, and these observations led to a search for the impact of such peptides on PTH physiology and assay. This non–(1-84) PTH appeared to represent 12% to 25% of total intact PTH immunoreactivity and appeared to be regulated in a similar fashion to PTH (1-84).[14] Because the 2-site intact PTH assay did not recognize large PTH fragments such as PTH (39-84), or PTH (53-84), because these would not be recognized by the detection antibody, and it did not react with PTH (1-34), because this was not captured by the capture antibody, it was proposed that the non–(1-84) PTH peak was a large PTH fragment with a sufficiently preserved N-terminal structure to allow interaction with the detection antibody. Subsequent experiments with synthetic PTH (7-84) indicated that this peptide eluted in a similar position on HPLC and was detectable in the 2-site intact immunometric assay.[19] Thus, it is likely that the non–(1-84) PTH peak represents a PTH peptide similar to, if not identical to, PTH (7-84). It subsequently was shown that this non–(1-84) PTH fragment accumulated in renal failure in a similar fashion to other C-terminal fragments, and in patients with chronic renal failure, the non–(1-84) PTH could represent 35% to 55% of intact PTH immunoreactivity. This amount, however, only would represent approximately 1% to 2% of total C-terminal immunoreactivity under the same circumstances[14] (Fig 1).

These observations then led to the refinement of the assay technique, such that the detection antibody for a 2-site immunometric assay was directed toward the precise N-terminus of the PTH molecule, and because of the specificity of this detection antibody for the very N-terminal end of the PTH molecule, this PTH immunometric assay showed no cross-reactivity with PTH (7-84), and thus appeared to be specific for intact PTH (1-84).[20-22] Consistent with the characteristics of this assay, this second-generation PTH immunometric assay detected only a single species of intact PTH on HPLC fractionated serum samples whereas the older immunometric assay revealed 2 peaks of PTH immunoreactivity.[21]

Correlations between first-generation immunometric assays and second-generation assays specific for PTH (1-84) are highly significant in all clinical circumstances and, in general, it appears that assays specific for PTH (1-84) give values 50% to 60% of those achieved with the first-

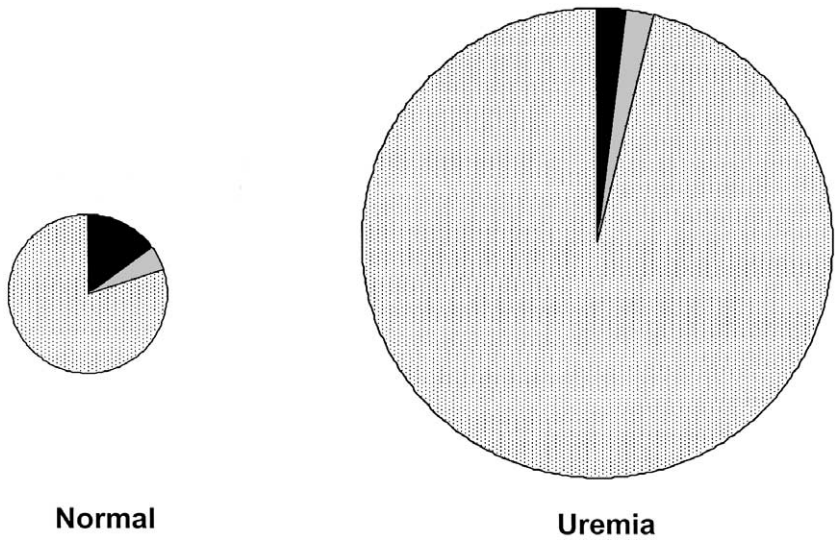

Fig 1. The proportions of PTH (1-84) and PTH ("7-84") in relation to the total circulating midregion/C-terminal immunoreactive PTH in normal and uremic patients' plasma. In normal subjects, PTH 1-84 (black) plus PTH ("7-84") (grey) together account for approximately 20% of total immunoreactive PTH peptides and PTH ("7-84") represents approximately 25% of PTH (1-84) concentration. In uremia, total immunoreactive PTH peptides are increased markedly owing to accumulation of PTH fragments. PTH (1-84) and PTH ("7-84") are approximately equal in concentration but together only represent 2% to 4% of total circulating PTH peptides. ■, 1-84; [GREY], PTH "7-84"; [DOTS], Mid/C PTH.

generation immunometric assays (Fig 2). Any diagnostic advantage of the second-generation immunometric assays over the predecessor appears to be slight at the present time. However, additional advantages may be relevant in that the second-generation immunometric assays may offer improved standardization of results between different laboratories throughout the world because current intact assays have not been standardized for the ability to detect the non–PTH (1-84) peptide(s), perhaps explaining the differences in results obtained with standard plasma samples in clinical laboratories. This may be extremely relevant to the field of renal osteodystrophy as the appropriate range for desired target PTH values becomes refined. An example of the excellent correlation between 2 assays specific for PTH (1-84) and the virtually identical results obtained is shown in Figure 3.

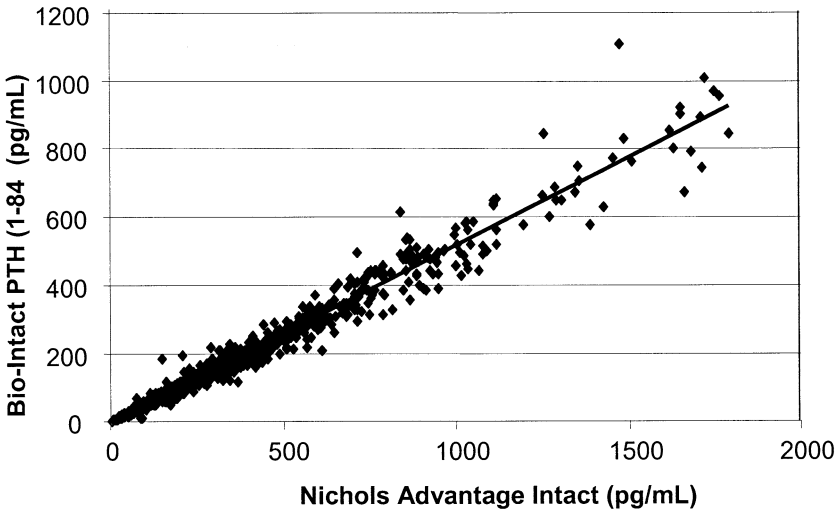

Fig 2. Correlation between a PTH assay specific for PTH (1-84) (Bio-Intact PTH; Nichols Institute Diagnostics, San Clemente, CA) and the older intact PTH assay that detects both PTH (1-84) and PTH ("7-84"). The results are highly correlated with PTH (1-84) values being approximately 50% of the older assay. (Personal communication, Dr. K. Ramki, Nichols Institute Diagnostics, San Clemente, CA.) y = .52 × −2; R^2 = .97; n = 995.

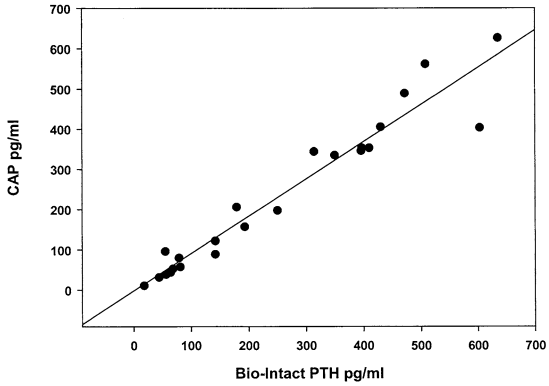

Fig 3. Values for PTH (1-84) in patients on hemodialysis measured in 2 different PTH assays, both of which are specific for PTH (1-84). CAP (cyclase activating PTH) (Scantibodies, Santee, CA) and Bio-Intact PTH (Nichols Institute Diagnostics). The values are highly correlated and the results are virtually identical. These observations support the potential for excellent standardization with such specific assays. R = .97; Y = .92 × −1.75; N = 23.

The difference between the values obtained with the first-generation and second-generation immunometric assays can, by subtraction, give a value for the non–(1-84) PTH circulating. This can, therefore, provide an estimate of the amount of such fragments that circulate without the necessity for HPLC fractionation.

The as yet not definitively characterized non–(1-84) PTH peptide(s), presumably PTH (7-84), recently has attracted considerable interest because of several observations both in vivo in experimental animals and in vitro that have suggested that there may be hitherto unrecognized biologic activities attributable to such PTH peptides. Thus, in the rat in vivo, parathyroidectomy leads to a decrease in blood calcium levels and administration of PTH (1-84) can increase serum calcium levels.[23,24] However, it was shown that if PTH (7-84) was given to the animal together with PTH (1-84), the calcemic effect of PTH (1-84) was abolished. It was further recognized that PTH (7-84) given alone resulted in a decrease in serum calcium levels, suggesting independent biologic activity attributable to this PTH peptide. These observations, shown by 2 groups of investigators, suggest the possibility that circulating non–(1-84) PTH may potentially have significant biologic effect by opposing the calcemic actions of PTH (1-84). If indeed PTH (7-84) has significant biologic actions, then it is necessary to understand the mechanisms of action and to define with which receptors this PTH peptide is interacting. PTH (7-84) does not bind to the PTH-1 receptor in kidney-derived cells or in osteoblast-like cells.[25-27]

RECEPTORS FOR PTH

The receptors for PTH peptides are listed in Table 1.

The classic biologic actions of PTH are mediated by binding to the type 1 PTH receptor, also known as the common receptor for PTH and PTH-related peptide (PTHrP), which has been cloned and characterized.[28] The binding of PTH to the PTH-1 receptor is mediated by the N-terminal region of the PTH (1-84) peptide. This is also the case for PTHrP, which is identical to PTH for 8 of the first 13 N-terminal amino acids. These 2 peptides are equivalent in their ability to activate the PTH receptor. Several sites of importance in the binding of PTH to this receptor have been shown. Thus, it has been shown that modifications of the amino acid composition of the N-terminus of PTH or deletions of a few amino acids at the N-terminus substantially diminishes the ability of such peptides to bind to the PTH-1 receptor, whereas similar deletions at the C-terminal end of PTH do not appear to affect receptor binding.[29] Thus, PTH (1-34) and PTH (1-84) are equivalent in terms of

Table 1. Receptors for PTH Peptides

Receptor	Ligands	Distribution	Actions
		Bone kidney	*Classic actions of PTH*
	1-84	Vasculature	
PTH1R	1-34	Liver	↑ Ca, ↓ P
	PTHrP	Lung	↑ Bone turnover
		Other	
	TIP 39	CNS	
PTH2R	1-34	Pancreas	?
	1-84	Testis	
		Placenta	
PTH3R	PTH	Zebra fish	?
	PTHrP		
	1-84		
	7-84		
	19-84	Bone	↓ Bone resorption
C-PTHR	24-84	?Kidney	↓ Osteoclast generation
	35-84		
	39-84		
	53-84		

NOTE. ↑, increased; ↓, decreased.

ligand-binding affinity for the PTH-1 receptor and the ability to initiate receptor signaling. All of the classic actions of PTH appear to be mediated through this receptor.

Additional PTH receptors also have been identified. The PTH-2 receptor, originally isolated from the cerebral cortex, and that appears to be expressed throughout the nervous system, binds PTH (1-34) but not PTHrP.[30] Recent observations indicate that the endogenous ligand for this receptor is tuberoinfundibular protein, TIP-39, however, the functional role for this PTH-2 receptor remains unclear.[31] An additional PTH receptor, the PTH-3 receptor, has been identified in zebra fish, but little is known about this receptor at the present time.[32]

Relevant to the recent observations regarding potential biologic actions of PTH that may be attributed to non–(1-84) PTH, are data reported in the past few years suggesting that there may be yet another PTH receptor, this one having binding specificity for the C-terminal region of PTH.[26,33-36] Such a receptor has been shown by radioligand binding studies in osteoblast-like cells and in cells resembling osteocytes but has not yet been cloned and has been called C-PTH receptor.[26,37] High-affinity radioligand binding can be shown using PTH 19-84, which does not bind to the PTH-1 receptor and can be competed effectively by PTH (1-84), PTH (24-84) and PTH (39-84). The presence of such a receptor shown by radioligand binding studies has taken on greater significance in view of the additional observations of Divieti et al,[38] who showed the presence of these receptors on osteocyte-like cells and went on to show functional effects of PTH (7-84) in vitro. Thus, these investigators have shown that PTH (7-84) can inhibit calcium release from prelabeled neonatal mouse calvariae, both in the basal state and when bone resorption is stimulated by PTH (1-84), PTH (1-34), calcitriol, prostaglandin E, or interleukin 11. Thus, PTH (7-84) appears to be an inhibitor of basal and stimulated bone resorption and it appears that these effects occur independently of PTH. These investigators have further shown that PTH (7-84) and PTH (39-84) appear to inhibit the formation of osteoclast-like cells in murine bone marrow cultures.[38] Thus, PTH (7-84) and other large C-terminal fragments may have a role in regulating osteoclastogenesis by acting through a C-terminal PTH receptor. The observation by radioligand binding studies that this receptor may be expressed in high levels in osteocytic-type cells, suggests an additional target for such PTH-derived peptides and may potentially impact on osteocytic osteolysis. The existence of such a receptor conceivably could explain the observations of blunting of the calcemic effect of PTH (1-84) by PTH (7-84) seen in experimental animals.[23,24]

IMPLICATIONS OF RECENT FINDINGS

The observations of a receptor for C-terminal regions of PTH with potential biologic actions opens a new area of research in parathyroid physiology, such that the possibility exists that a true index of PTH activity may require an integrated assessment of the actions of PTH at the PTH-1 receptor as well as any actions of PTH peptides at the C-terminal PTH receptor, which might have a biologic response that is opposite in direction. Thus, the balance between the positive effects of PTH might need to be weighed against the opposing actions of PTH and PTH peptides at the C-terminal receptor.[39-41] In this regard, then it is necessary to understand the regulation of non–(1-84) PTH concentrations in plasma. Studies by several investigators have shown in the past that the production of PTH fragments within the parathyroid glands is a calcium-regulated process, and thus more PTH fragment generation occurs in the parathyroid gland in states of hypercalcemia.[42,43] Because such fragments are secreted from the parathyroid gland, in addition to intact hormone, then in hypercalcemia the ratio of intact PTH to non–(1-84) PTH fragments would decrease.[44] This has been shown to be the case by several investigators.[24,45] Thus, the interpretation of results of measurements of such non (1-84) PTH peptides in blood would require a knowledge of the ambient calcium concentration for correct interpretation. Other factors also potentially might influence the relative amounts of PTH (1-84) and PTH fragments in the circulation and therefore much needs to be learned before clinical interpretations of a PTH/fragment ratio are on firm ground (Table 2).

An additional consideration might be that the production of excessive amounts of non–(1-84) PTH fragments in relation to PTH (1-84) might have an influence on the state of bone turnover, and in this regard, it has been suggested that the utilization of a ratio of intact PTH to non–(1-84) PTH fragment may be valuable in determining bone turnover noninvasively in patients with renal fail-

Table 2. Known and Unknown Factors in the Regulation of the PTH/non–(1-84) PTH Fragment Ratio in Patients With Advanced Kidney Failure

The influence of serum calcium
What is the influence of serum phosphorus?
What is the influence of the type of phosphate binder?
Does calcium load affect the ratio?
What is the effect of vitamin D sterols with and without changes in serum calcium levels?
Do calcimimetic agents affect the ratio?
Is there an effect of the level of expression of the parathyroid calcium receptor?
Is there an effect of parathyroid mass?
Is there an influence of race, age, or sex?
How reproducible is the ratio over time?
What are the correct fragments to measure?

ure on hemodialysis.[45] Other investigators have not been able to confirm this and considerable controversy exists with this hypothesis at the present time.[46-50] The reasons for this controversy need to be evaluated carefully and much further work must be performed before the clinical utility of this approach can be clarified. It is possible that experimental differences, different patient populations, and other covariates explain the different results by the different investigators, and as we begin to understand the biology and actions of PTH fragments at the C-PTH receptor and the regulation of such actions, it is likely that the explanation will become clear. An additional consideration is to understand the actions of other PTH fragments that circulate in very high concentrations in renal failure for their potential actions at this receptor, and, as we understand which fragments can interact with the C-PTH receptor and learn more about their biologic actions of the C-PTH receptor, it may be that additional assays may need to be developed to properly assess the appropriate spectrum of biologic activity. Only when these aspects are understood will we be able to utilize the ratio of PTH to non–(1-84) PTH fragments to define the clinical utility of such measurements. In the meantime, the improved assays for PTH (1-84) should provide the means to obtain meaningful target therapeutic ranges that are reproducible across different laboratories throughout the world for the control and treatment of secondary hyperparathyroidism.

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