Circulating parathyroid hormone (PTH) regulates normal bone and mineral ion homeostasis and is central in the pathogenesis of bone disease in primary and secondary hyperparathyroidism, especially in advanced renal insufficiency (1). The major biological effects of PTH, a linear polypeptide 84 amino acids in length, reflect activation of the G-protein-coupled PTH/PTHrP receptor (PTHR) expressed on target cells in the renal tubules and the osteoblastic lineage of bone (2). Activation of PTHRs requires only the most N-terminal portion of PTH [PTH(1–34) is a full agonist] and is mostly eliminated if the N-terminal serine residue of the human hormone is removed.

PTH, secreted by the parathyroid glands primarily in response to changes in blood ionized calcium, undergoes rapid endopeptidic cleavage, mainly by Kupffer cells in the liver (3). This event generates a series of N-truncated “CPTH” fragments (the corresponding N-terminal segments are destroyed in situ) that reenter the circulation and are cleared mainly by glomerular filtration, an important route for PTH clearance as well (4). Chemical analysis of PTH fragments isolated from rat blood after administration of radiolabeled bovine PTH established that hepatic metabolism yields a family of CPTH fragments with NH2 termini ranging between residues 34 and 43, although the methods used likely would not have detected substantially larger CPTH fragments (5). Interestingly, chemically similar CPTH fragments are secreted directly from the parathyroid glands as well, and the ratio of secreted CPTH fragments to intact PTH increases as blood calcium increases (6, 7). CPTH fragments typically circulate at a three- to fivefold molar excess over intact PTH, but this ratio may increase dramatically in end-stage renal disease (ESRD), depending on the severity and chronicity of renal insufficiency and the blood concentrations of calcium, phosphate, and 1,25-dihydroxyvitamin D, all of which regulate PTH secretion (1, 8).

Measurement of circulating biologically active PTH is crucial for the differential diagnosis of hypercalcemia and hypocalcemia and in the diagnosis and management of primary hyperparathyroidism, vitamin D deficiency, and renal osteodystrophy. Early efforts to measure PTH by use of single-antibody RIAs directed against the more immunogenic C-terminal region of PTH were confounded by the high and variable amounts of reactive CPTH fragments, especially in ESRD, in which results of PTH assays correlated poorly with bone histology (9). In fact, observations that RIAs based on different antisera produced divergent results had provided early clues to the surprising heterogeneity of circulating PTH peptides (10). This problem appeared to be solved with the advent of “intact PTH” two-site IRMAs, which typically use a solid-phase capture antibody directed against the PTH COOH terminus [usually PTH(39–84)] and a labeled detection antibody raised against PTH(1–34) (11) (see Fig. 1). These IRMAs thus were “blind” to the major “short” CPTH fragments previously identified in blood and reacted exclusively with peptides long enough to incorporate both C- and N-terminal PTH sequences, the only known candidate for which was intact PTH(1–84). The clinical utility of these first-generation IRMAs in the diagnosis of primary hyperparathyroidism and in monitoring the severity of secondary hyperparathyroidism in ESRD is well established. On the other hand, it is known that serum concentrations of intact PTH measured in this way tend to overestimate the severity of PTH-related bone disease seen on biopsy in ESRD and that a decrease in intact PTH concentrations to normal in this setting, as with calcium and active vitamin D analog therapy, is associated with adynamic bone disease (1). This discrepancy was attributed to the resistance of bone to PTH in ESRD, and nephrologists circumvented the problem simply by targeting intact PTH to concentrations two- to fourfold above normal (1, 12).

All seemed well until 1996, when Brossard et al. (13) reported that up to one-half of the signal detected by intact PTH assays in blood from dialyzed ESRD patients is attributable to circulating forms of PTH that can be separated from hPTH(1–84) by reversed-phase HPLC. These peptides, collectively termed “non-(1–84) PTH”, subsequently were found not to react in new, second-generation PTH IRMAs, in which the recognition epitope of the detection antibody is located at the extreme NH2 terminus of PTH (14). Thus, peptides lacking the N-terminal serine of hPTH fail to register in these new assays, whereas the detection antibodies used in the first-generation IRMAs bind N-truncated peptides, such as PTH(7–84), just as well as those with an intact NH2 terminus (14, 15). The precise chemical structures of these N-truncated non-(1–84) PTH fragments remain unknown, although they are long enough to react with both C- and N-directed antibodies and eluted similarly to synthetic hPTH(7–84) in the original HPLC systems used (16).
Non-(1–84) PTH peptides constitute roughly 15% of the intact PTH signal in normal plasma but 30% or more in primary and secondary hyperparathyroidism. Like other CPTH fragments, they are both secreted by the parathyroids and generated by peripheral metabolism of the hormone (17, 18). When administered to thyroparathyroidectomized rats, hPTH(7–84), a model for these non-(1–84) PTH peptides, antagonizes the calcemic effect of co-infused PTH, although it does not effectively bind to or antagonize the PTHR (17, 19). In thyroparathyroidectomized rats with moderate renal failure, hPTH(7–84) infused continuously for 2 weeks antagonizes the increase in both bone turnover and serum calcium otherwise elicited by co-infused hPTH(1–84) (20). In vitro, hPTH(7–84) inhibits osteoclast formation induced by a variety of bone-resorbing agonists and binds to a novel class of PTH receptors on bone cells (CPTHRs), distinct from PTHRs, that recognize determinants located C-terminal to the N-terminal domain needed for PTHR activation (21, 22). These observations have introduced the possibility that CPTH fragments, including non-(1–84) PTH peptides, may not be biologically inert, as previously assumed, but instead may act through receptors on bone cells, different from PTHRs, to modulate regulation of skeletal turnover by PTH or other agents.

Currently, manufacturers are offering measurements of full-length, or “whole-molecule”, PTH based on second-generation IRMAs and, on subtracting this value from that obtained with a first-generation intact PTH IRMA, an estimate of the residual non-(1–84) PTH. The added clinical value of this approach remains uncertain, however, because results of first- and second-generation IRMAs generally are closely correlated in both primary and secondary hyperparathyroidism (14, 17). Some have found that bone histology (the rate of bone turnover) in ESRD correlates well with the ratio of PTH to non-(1–84) PTH, as might be expected if non-(1–84) PTH fragments, presumably acting via CPTHRs, antagonize the skeletal actions of PTH (mediated by PTHRs) (23). Others have been unable to successfully predict bone histology using this approach, however (24, 25). Use of new second-generation IRMAs intraoperatively to monitor the decrease in circulating PTH that signals successful parathyroidectomy might be expected to be superior to first-generation assays, which also detect the more slowly cleared non-(1–84) PTH fragments. The new IRMAs may offer some advantage here, but the improvements noted to date have been modest (26). Similarly, although one might expect that specific measurement of bioactive, full-length PTH would improve diagnostic accuracy in mild primary hyperparathyroidism, in which increased serum calcium may coexist with normal intact PTH or vice versa, the observed advantages of second-generation IRMAs in diagnosing primary hyperparathyroidism have been marginal to date (14).

The novel findings reported by D’Amour et al. (27) in this issue of Clinical Chemistry add new understanding, and yet more complexity, to this already unsettled sce-
D’Amour et al. (27) is limited to analysis of seven healthy individuals, five patients with primary hyperparathyroidism, and eight pools of renal failure serum. It may be that the concentrations of amino PTH, like those of non-(1–84) PTH (14), will be found in larger patient populations to vary much more widely, relative to unmodified PTH, than has been observed to date. Perhaps this will help to explain why second-generation IRMAs have not yet demonstrated dramatic clinical advantages over first-generation assays. Ultimately of course, the clinical importance of distinguishing the newly described amino PTH from authentic unmodified PTH will be dictated by what is yet to be learned of its bioactivity, structure, source, and regulation. Certainly, if amino PTH turns out to represent a phosphorylated form of the hormone, it should be possible to develop specific antibodies to aid in its recognition and/or extraction.

What is very clear is that the chapter has not yet closed on the analysis and biological significance of the various circulating forms of PTH. The prospect of multiple full-length and C-terminal species of the hormone with potentially independent regulation and biological properties predicts an exciting new episode in PTH research. As investigators continue to peel back the layers of this onion, it is likely that we ultimately will arrive at the kernel(s) of biologically active PTH most relevant to the clinical manifestations of primary or secondary hyperparathyroidism. Meanwhile, clinicians will continue to rely on those assays, as imperfect as they may be, with which they have developed the most extensive familiarity and clinical experience.

References

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