**Parathyroid Hormone, A Uremic Toxin**

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**ABSTRACT**

Despite the innovations in the treatment of secondary hyperparathyroidism, there are uremic patients with marked elevation in PTH levels. Uremic toxicity is in part attributable to the excess of circulating PTH. It has been known for many years that PTH may induce changes in cell calcium, a key intracellular signal required for normal cell function. The effect of PTH in dialysis patients is not limited to bone; the diversity of biologic effects of PTH is summarized in this review. In addition, the present review addresses other issues: (i) the presence of different circulating PTH fragments in uremic patients, (ii) the PTH assays currently utilized to measure circulating PTH, and (iii) the fact that some of the PTH effects seen in uremic patients may be due to the interaction of C-terminal PTH fragment with putative C-terminal PTH receptors.

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**Secondary Hyperparathyroidism**

Secondary hyperparathyroidism is a universal complication of chronic renal failure. Hyperplasia of the parathyroid glands is typically seen in these patients and it starts to develop at very early stages of renal disease. Decreased production of calcitriol, accumulation of phosphate, and a tendency to hypocalcemia promote an increase in parathyroid hormone (PTH) secretion and synthesis as well as an increase in parathyroid cell proliferation. As the disease progresses, there is a decrease in the number of vitamin D receptors (VDR) and calcium sensing receptors (CaR) in the parathyroid glands. Thus, extracellular calcium and calcitriol are not able to control parathyroid cell function. The rate of parathyroid cell proliferation increases progressively and clonal proliferation is frequently observed in advanced stages of parathyroid hyperplasia. The expression of VDR and CaR are further reduced in nodular (monoclonal) parathyroid hyperplasia; then, control of hyperparathyroidism becomes difficult (1,2). Furthermore, in renal failure, there is a resistance to the action of PTH which may be due, at least in part, to diminished phenotypic expression of PTH receptors and to the high serum phosphate levels. However, in some patients, the excessive amount of PTH secreted overcomes bone resistance to PTH and patients develop high bone turnover, histologically defined as osteitis fibrosa.

**Skeletal Effect of PTH: Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)**

The term Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) has been introduced to describe a broad clinical syndrome that develops in uremic patients as a systemic disorder, manifested by disturbances in mineral and bone metabolism and extracellular calcification. As part of this syndrome, patients develop various types of bone disease known as renal osteodystrophy (ROD). (3). Bone biopsy and appropriate histomorphometric analysis allows the identification and classification of the different types of ROD. The types of renal bone disease are classified as high bone turnover, low bone turnover, and the combination of low and high turnover. Osteitis fibrosa, observed in patients with high PTH levels, characterizes high bone turnover disease; bone turnover is abnormally high; a portion of the calcified bone loses its lamellar structure and appears as woven bone. There is resorption of cortical bone and marrow fibrosis.

Osteomalacia and adynamic bone disease represent low bone turnover. In osteomalacia, the bone surface is covered with uncalcified osteoid and cell activity is decreased. Mineralization deficiency is demonstrated by the lack of tetracyclin uptake. Osteomalacia was common in patients with aluminum intoxication or severe vitamin D deficiency. Adynamic bone disease is also characterized by low bone turnover; both bone resorption and bone formation are decreased, but in this case osteoid is almost absent. Mixed bone disorder possesses characteristics of both high and low turnover (4).

These various types of ROD are due to different degrees of hyperparathyroidism, treatment with vitamin...
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of red blood cells and this may affect their integrity (7). All patients share a common factor, the presence of the uremic condition.

Extraskeletal Effects of PTH: Contribution of High PTH to the Uremic Syndrome

In addition to the effects on mineral metabolism, markedly elevated PTH may behave as “uremic toxin” responsible for some of the abnormalities of the uremic state. During the last decades, PTH has been implicated as a pathogenic factor of many alterations present in uremia. Briefly, we will review the potential adverse effects of secondary hyperparathyroidism in patients with chronic kidney disease (CKD).

Parathyroid hormone produces an increase in “intracellular calcium” (5,6) in many different cell types with the exception of smooth muscle cells. The increase in cytosolic calcium levels seems to be caused by both an increased influx and a decreased efflux of calcium from the cell. PTH activates the l-type calcium channels by a process that can be blocked by PTH antagonists. It appears that PTH plays an important role in some of the abnormal electroencephalographic patterns observed in uremia. This may be due to a potential role of PTH in increasing calcium content of brain. Parathyroid hormone also has been implicated as a pathogenic factor in many other alterations present in uremia, i.e., peripheral neuropathy, carbohydrate intolerance, hyperlipidemia, and other alterations. Unfortunately, outstanding clinical research is lacking in this field and conclusive experimental data are practically nonexistent.

High blood levels of PTH increase the calcium content of red blood cells and this may affect their integrity (7). Both the amino-terminal (1–34) PTH and the intact (1–84) PTH increase the “osmotic fragility of erythrocytes”; this may explain reduced erythrocyte survival in uremic patients. PTH caused significant influx of Ca45 into RBC; this action of PTH required extracellular calcium, was mimicked by calcium ionophore, and was partially blocked by verapamil. Other studies have shown that PTH decreases erythropoiesis directly by causing bone marrow fibrosis (8).

The effect of high PTH levels on isolated “heart” mitochondria has been evaluated. PTH inhibited mitochondrial respiration, reduced phosphorylation, and uncoupled oxidative phosphorylation. These adverse effects required extracellular calcium and were dose dependent. The reduction of oxidative phosphorylation may result in decreased ATP synthesis. These events may cause long-term adverse effects of PTH on the myocardium (9). An inhibitory effect of PTH on energy production and utilization was also observed in skeletal muscle (10). Verapamil treatment reversed all these adverse effects of PTH. Thus, it has been suggested that PTH may be partly responsible for the muscular dysfunction and wasting in uremic patients with secondary hyperparathyroidism. In hemodialysis patients, a negative effect of high PTH levels on left ventricular myocardial function and cardiac hypertrophy was shown by Druke et al. (11) and London et al. (12), respectively. In uremic rats, Amann et al. (13) demonstrated a stimulatory effect of PTH on cardiac fibrosis. In a recent in vitro study, PTH had no effect on vascular smooth muscle cell (VSMC) proliferation or number, but it increased production and reorganization of collagen by VSMCs (14).

“Peripheral neuropathy” is a common complication of chronic uremia. The effect of PTH and uremia on motor-nerve conduction velocity and nerve calcium content has been evaluated in experimental animals (15). Calcium content in peripheral nerve was increased in uremic animals and in animals with normal renal function receiving parathyroid extracts. Thyroid-parathyroidectomy (TPTX) prevented the increase in nerve calcium content induced by uremia and discontinuation of administration of parathyroid extracts restored peripheral nerve calcium to normal. The increments in peripheral nerve calcium were associated with slowing of motor-nerve conduction velocity. It decreased after 3 days of uremia in animals with intact parathyroid glands and TPTX before induction of renal failure prevented the fall in MNCV. A retrospective analysis in nondiabetic uremic patients showed that the impairment of motor-nerve conduction velocity was more severe in those patients with the highest PTH levels (16).

Experimental work in uremic animals demonstrated that high PTH was responsible for “glucose intolerance” associated to uremia. Studies in uremic dogs with and without intact parathyroid glands showed that PTH is responsible for glucose intolerance in uremia; excess PTH in CRF interferes with the ability of the beta-cells to augment insulin secretion appropriately. The effect of high PTH levels on glucose metabolism has also been demonstrated in humans (17). Hyperparathyroidism might be involved in “other hormonal abnormalities” associated to uremia: increased aldosterone secretion, decreased serum testosterone levels and increased circulating levels of prolactin (18).

In vitro, PTH exerts a direct effect on lymphocyte and polymorphonuclear leukocyte (PMNL) function. Thus abnormalities of the “immune system” in chronic uremia may be in part related to the degree of secondary hyperparathyroidism. An acute effect of PTH on T lymphocytes results in an increase in cell proliferation and cytokine production. Chronic exposure to PTH causes the opposite (19). In dialysis patients, production of immunoglobulins by B lymphocytes is decreased; addition of PTH to the medium reduced the immunoglobulin production by B cells from normal subjects and dialysis patients. Phagocytosis is significantly impaired in dialysis patients, but it may be normalized with verapamil; similarly, in uremic rats, parathyroidectomy (PTX) or verapamil improve PMNL function (20).

In renal failure rats elevated plasma “triglyceride levels” were normalized after PTX or treatment with verapamil which implicates PTH as a pathogenic factor in disturbances of lipid metabolism in uremia. Tissue lipoprotein lipase is downregulated in chronic renal failure rats which can be prevented by PTX or verapamil (21).
**PTH Structure and Metabolism**

Plasma PTH consists of a mixture of intact PTH (1–84) also called “whole PTH,” a single-chain polypeptide of 84 amino acids, and smaller molecular weight carboxy-terminal fragments (C-PTH). The hormonal fragments arise from metabolism of 1–84 PTH by peripheral organs as well as from secretion of C-PTH fragments from the parathyroid glands. Structural requirements for the known biologic actions of PTH reside in the amino-terminal portion of the PTH molecule; more recent studies have suggested the presence of specific receptors for C-PTH.

Parathyroid cells secrete both PTH (1–84) and C-PTH (22). The C-PTH fragments may also be generated by liver cells from proteolysis of PTH (1–84). The C-PTH fragments are subsequently removed by the kidneys. Thus, impairment of renal function causes accumulation of C-PTH. Amino-terminal fragments (N-PTH) are produced by Kupffer cells during cleavage of I-PTH but, unlike the corresponding C-PTH fragments, are rapidly degraded.

**Regulation of PTH (1–84) and PTH Fragments**

Although hypercalcemia reduces the secretion of both PTH (1–84) and C-PTH, the reduction of PTH (1–84) secretion is more marked than that of C-PTH (22). The clearance of C-PTH from plasma has been studied in the rat (23). In normal rats radioiodinated C-PTH was extracted by kidneys (33%), muscle (16%), bone (7%), liver (3%), and other tissues (1%). In nephrectomized rats, 25% of C-PTH fragments were cleared in muscle, 10% in bone, and 7% in liver, with less than 1% in other tissues. Thus, nonrenal tissues can increase their ability to remove C-PTH fragments in renal failure.

In dialysis patients there are large amounts of circulating amino-terminal truncated C-PTH fragments which are long enough to be detected by the conventional two-site immunoassays for PTH called “intact PTH assays.” However, these assays detect C-PTH fragments lacking few amino acids of the amino-terminal portion which are required for bioactivity. In fact, the presence of the amino-terminal serine is necessary for binding (and subsequent biologic effect) of PTH to its own PTH receptor (PTH1R). In renal failure the large amino-terminal truncated C-PTH fragments may constitute up to 50% or more of the total PTH immunoreactivity. Thus, the conventional “intact PTH assays” measure both the PTH (1–84) molecule plus large amino-terminal truncated C-PTH fragments (24). Now, the term “bioactive PTH” or “whole PTH” is being used by different authors to name the entire PTH molecule: PTH (1–84).

**PTH Immunoassays**

First generation radioimmunoassays for PTH (RIA) used antibodies against Carboxy (C) and Amino (N) terminal PTH fragments (Figs. 1A and B). These assays detected all circulating PTH fragments including whole PTH (1–84). In 1987, “Nichols Institute Diagnostic, Inc.” developed an IRMA assay using two antibodies, one against the N-terminal part of the molecule (1–34 epitope) and a second one against the C-terminal part of the PTH molecule (39–84) (second generation assays). This assay was said to detect the intact PTH molecule, but, as mentioned before, it also detected some large C-terminal fragments (Fig. 1C). Clinical studies showed that PTH measured by this assay correlated with bone hystomorphometric parameters; for many years the intact PTH assay (IRMA) was considered the gold standard for PTH measurement. Thereafter, various intact PTH kits (IRMA or quimioluminescence assays) became commercially available. Antibodies used by the different kits are not identical and also the standards used by the different companies are not the same. Thus, same serum samples measured with different assays resulted in wide differences in PTH values (25). This fact has important clinical implications because the recommended target PTH (K/DOQI guidelines) may vary according to the PTH assay employed.

More recently, it has been possible to generate antibodies against the first 4–5 amino acids of N-terminal PTH molecule, which in combination with the C-terminal antibody allows the detection of “whole PTH” or “bioactive PTH” (third generation assays) (Fig. 1D). To date, there are no clinical studies which correlate whole PTH levels with bone hystomorphometric parameters.

**PTH Receptors**

The classical actions of PTH on kidney and bone are well established. The N-terminal (1–34) is required for activation of adenylyl cyclase and the C-terminus (i.e., residues 15–34) for high-affinity receptor binding. The PTH receptor (PTH1R), coupled to G proteins, is activated equivalently by 1–84 PTH, N-terminal PTH, and the PTH related peptide (PTHrP). The PTH1R is highly expressed in bone and kidney, but is found also in a variety of tissues not regarded as classical PTH target tissues. This explains the widespread effects of PTH and illustrates the reasons to consider PTH as a clinically relevant uremic toxin in patients with advanced renal disease. Another PTH receptor, the PTH2R, is mainly expressed in central nervous system, thyroid, gastrointestinal tract, pancreatic islet cells, and the cardiovascular system. However, the PTH2R is not expressed in renal tubules and bone and is not activated by PTHrP. Like the PTH1R, the PTH2R responds to PTH, with generation of cAMP and increase in intracellular calcium.

A number of experiments revealed that high concentrations of the biologically active N-terminal fragment PTH (1–34) could only partially displace the binding of intact hormone (PTH 1–84), which implied that not all of the binding of intact hormone to target tissues
involved the receptor for the N-terminal 34 residues of PTH and suggested the presence of binding to C-PTH. In the kidney, McKee and Murray (26), using PTH (1–34) and PTH (53–84) demonstrated a N-terminal binding site and a C-terminal binding site; the C-terminal site had lower affinity and did not activate adenyl cyclase. Similar findings have been obtained in bone cells. The breeding of mice without expression of PTH1R helped to demonstrate the presence of C-PTH receptors: Divieti et al. (27) immortalized clonal cell lines from these mice and demonstrated the presence of C-PTH receptors.

Fig. 1. The different types of PTH assays. First generation assays (A and B) used antibodies against C-terminus or N-terminus. The second generation assays (C) used two antibodies directed against the C-terminus and different sequences of the amino terminus PTH. The third generation assays (D) used antibodies against the C-terminus plus antibodies directed against aminoacids 1–4 of N-terminus to assure a minimal or absent contamination of the large C-terminal fragments.

Fig. 2. Schematic representation of the biologic effect of activation of PTH1R and C-terminal PTH receptor.
PTHR-null mice with high expression of specific binding sites for 19–84 PTH fragment; they were able to identify that the sequence (24–27) and (55–84) were important determinants of binding affinity (48–49). Interestingly, the binding of the C-PTH fragment increased alkaline phosphatase activity in ROS 17/2.8 osteosarcoma cells and the action of PTH (1–34) resulted in a decrease in alkaline phosphatase activity.

The Role of C-PTH Fragments

Large C-PTH fragments such as PTH (7–84) are markedly increased in uremic patients. Slatopolsky et al. (28) performed experiments to evaluate the role of PTH (7–84) in the regulation of calcium. The administration of PTH (7–84) to previously parathyroidectomized hypocalcemic rats produced a further decrease in serum calcium. Furthermore, the administration of PTH (7–84) prevented the rise in serum calcium otherwise induced by PTH (1–84) when this peptide was coadministered at an equimolar dose. The phosphaturic effect of PTH (1–84) was reduced approximately 50% by administration of a fourfold molar excess of PTH (7–84). Similar results were obtained by Nguyen-Yamamoto et al. (29) in thyrroparathyroidectomized rats receiving a continuous infusion of PTH (7–84) which decreased serum calcium and blocked the calcemic response to PTH (1–34) or PTH (1–84). The infused C-PTH fragments also lowered serum phosphate; as renal excretion of phosphate or calcium was not increased, the results should be interpreted as a decrease in fluxes of calcium and phosphate from bone. In vitro studies had demonstrated that the PTH (7–84) could not bind to PTHR1, therefore the observed in vivo effects of PTH (7–84) must be through the C-PTH receptors (Fig. 2).

Administration of PTH (7–84) by continuous infusion for 2 weeks to thyrroparathyroidectomized rats with renal failure antagonized the increase in bone turnover produced by a continuous infusion of PTH (1–84). In neonatal murine calvaria bones, the addition of PTH (7–84) lowered the basal rate of bone resorption as effectively as calcitonin and inhibited the resorption induced by PTH (1–34) and PTH (1–84). The decrease in calcium serum levels induced by administration of PTH (7–84) may be due to a decrease in osteoclast precursors which express C-PTH receptors (30). Thus, PTH (7–84) can inhibit bone resorption, at least in part by reducing the rate of formation of new osteoclasts.

Thus, during hypercalcemia the C-PTH receptors may help to maintain bone formation by decreasing bone resorption when C-PTH fragments levels exceed those of PTH (1–84). Taking into account the ratio of 1–84 PTH/C-PTH may be important to understand the changes in bone volume observed in dialysis patients. In this respect, the effects of C-PTH receptor activation by C-PTH fragments are somewhat opposed to the activation of PTH1R by the N-terminal system. Furthermore, the increased production of large C-PTH fragments by parathyroid glands during hypercalcemia may help to restore bone calcium by inhibition of bone resorption.

Conclusion

In chronic kidney disease, parathyroid hyperplasia with excessive production of PTH causes abnormalities in cell function contributing to uremic toxicity. High PTH causes increased bone turnover which is part of the “Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD)” manifested by disturbances in mineral and bone metabolism and extrasosseous calcification. PTH produces an increase in intracellular calcium and has been shown to cause osmotic fragility of erythrocytes, myocardial dysfunction and cardiac hypertrophy, peripheral neuropathy, glucose intolerance, increased aldosterone secretion, decreased serum testosterone levels, increased circulating levels of prolactin, abnormalities of the immune system, and disturbances of lipid metabolism. Most commercial PTH assays detect the entire PTH molecule (1–84) and large C-PTH fragments; values of PTH vary among the different PTH assays. There are experimental data which demonstrate biologic effects of C-PTH fragments that differ, and even oppose, from those observed with the PTH (1–84). Thus, the accumulation of C-PTH fragments in uremic patient add complexity to the biologic effects of the PTH system.

References