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Role of vitamin D metabolites in the regulation of PTH synthesis and secretion in chronic kidney disease

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ABSTRACT

Small decreases in serum Ca^{++} and more prolonged increases in serum phosphate (P), stimulate the parathyroid (PT) to secrete parathyroid hormone (PTH) and 1,25$(OH)_2$-vitamin D_3 decreases PTH synthesis and secretion. A prolonged decrease in serum Ca^{++} and 1,25$(OH)_2D_3$, or increase in serum P, such as in patients with chronic renal failure, leads to the appropriate secondary increase in serum PTH. This secondary hyperparathyroidism involves increases in PTH gene expression, synthesis and secretion, and if chronic, proliferation of the PT cells. A low serum Ca^{++} leads to an increase in PTH secretion, PTH mRNA stability and PT cell proliferation. P also regulates the PT in a similar manner. 1,25$(OH)_2D_3$ decreases PTH gene transcription and PT cell proliferation. 1,25$(OH)_2D_3$ increases the expression of the PT CaR and of the PT vitamin D receptor (VDR) which would amplify the effect of calcium and vitamin D to decrease PTH secretion and gene expression respectively. Hypocalcemia leads to increased PTH gene expression despite high serum 1,25$(OH)_2D_3$ levels due to an increase in calreticulin concentration in the PT nucleus. Calreticulin inhibited binding of the VDR complex to the PT vitamin D responsive element (VDRE) and inhibited the effect of 1,25$(OH)_2D_3$ on the PTH promoter. The role of less hypercalcemic analogs of 1,25$(OH)_2D_3$ in the management of patients with chronic kidney disease (CKD), together with calcimimetics and the judicious use of phosphate binding compounds is discussed.

INTRODUCTION

The action of 1,25(OH)$_2$D$_3$ or its analogues to decrease PTH secretion is now a well-established axiom in clinical medicine for the suppression of the secondary hyperparathyroidism of patients in chronic renal failure. So much so, that it is worthwhile to reflect upon its scientific basis.

Secondary hyperparathyroidism is a frequent complication in patients with CKD. The secondary hyperparathyroidism of chronic renal failure comprises increased PTH secretion, increased PTH synthesis, and increased parathyroid cell proliferation. Cellular and molecular studies have yielded insights and highlighted unanswered questions into these changes, particularly related to the regulatory actions of 1,25(OH)$_2$ vitaminD$_3$, calcium, and phosphate, which have important effects at all three levels of parathyroid dysfunction in CKD.

VITAMIN D AND THE PARATHYROID

Vitamin D deficiency was known to be associated with secondary hyperparathyroidism and this was always considered to result from decreased absorption of calcium from the diet with the resultant hypocalcemia stimulating the parathyroid to secrete more PTH[1]. The PT was not considered to be a target organ of vitamin D. With the discovery of 1,25(OH)$_2$-vitamin D$_3$ as the active metabolite of vitamin D it became feasible to test this accepted dogma in the laboratory. In vitro, using bovine PT cells in primary culture, we showed that 1,25(OH)-$_2$D$_3$ decreased PTH mRNA levels[2]. In in vivo studies we confirmed the physiological relevance of these in vitro studies[3]. 1,25(OH)-$_2$D$_3$ in physiologically relevant doses dramatically decreased the levels of PTH mRNA in the PTs of normal rats without changing the levels of serum calcium. We showed that the effect of 1,25(OH)-$_2$D$_3$ on the PTH gene expression was transcriptional[3].

We then showed that the 1,25(OH)-$_2$D$_3$ receptor mRNA is expressed at a very high concentration in the rat PT, similar to its concentration in the duodenum, the classical vitamin D target organ[4]. Moreover, the administration of 1,25(OH)-$_2$D$_3$ increased the levels of the 1,25(OH)-$_2$D$_3$ receptor mRNA in the PT, which would then increase the amount of receptor protein in the PT, and thereby amplify the effect of circulating 1,25 (OH)-$_2$D$_3$'s action on the PTH gene.

Further studies in humans confirmed these findings in patients with CKD[5]. 1,25(OH)-$_2$D$_3$ given to patients with CKD effectively decreases PTH secretion and the bone disease due to the high levels of circulating PTH. 1,25(OH)-$_2$D$_3$ also has an action to increase serum calcium and analogs of 1,25(OH)-$_2$D$_3$ were discovered that were less hypercalcemic than the natural compound. In many countries these compounds are now the mainstay of treatment of patients with CKD.

PROMOTER SEQUENCES

DNA sequences in the human PTH gene that bind the 1,25(OH)$_2$D$_3$ receptor (VDR) have been identified[6]. Nuclear extracts containing the VDR were examined for binding to sequences in the 5'-flanking region of the human PTH gene. A 25-bp oligonucleotide containing the sequences from -125 to -101 from the start of exon 1 bound nuclear proteins that were recognized by monoclonal antibodies against the VDR. The sequences in this region contained a single copy of a motif (AGGTTCA) that is homologous to the motifs repeated in the up-regulatory VDR response element (VDRE) of osteocalcin. When

placed upstream of a heterologous viral promoter, the sequences contained in this 25-bp oligonucleotide mediated transcriptional repression in response to $1,25(OH)_2D_3$ in GH4C1 rat pituitary cells but not in ROS 17/2.8 rat osteosarcoma cells. Therefore, this down-regulatory VDRE differs from up-regulatory VDREs both in sequence composition and in the requirement for particular cellular factors other than the VDR for repressing PTH transcription. Liu *et al*[7] have identified such sequences in the chicken PTH gene and demonstrated their functionality after transfection into the opossum kidney OK cell line. Selective mutations introduced into the element converted the negative activity imparted by the aPTH VDRE to a positive transcriptional response through[8].

The effect of $1,25(OH)_2D_3$ may involve heterodimerization with the retinoic acid receptor[9]. Moreover, combined treatment with $1x10^{-6}$ M retinoic acid and $1x10^{-8}$ M $1,25(OH)_2D_3$ more effectively decreased PTH secretion and preproPTH mRNA than did either compound alone[9]. Vitamin D receptor-retinoid X receptor (VDR-RXR) heterodimers induce DNA bending upon binding to various vitamin D response elements (VDRE) with or without a $1,25-(OH)_2D_3$ ligand[10] similar to other members of the steroid and thyroid nuclear receptor superfamily.

To determine what phenotypic abnormalities observed in vitamin D receptor (VDR)-ablated mice are secondary to impaired intestinal calcium absorption rather than receptor deficiency, Li *et al* normalized mineral ion levels by dietary means[11]. VDR-ablated mice and control littermates were fed a diet rich in calcium lactate that has been shown to prevent secondary hyperparathyroidism in vitamin D-deficient rats. This diet normalized growth and random serum ionized calcium levels in the VDR- ablated mice. The correction of ionized calcium levels prevented the development of parathyroid hyperplasia and the increases in PTH mRNA synthesis and in serum PTH levels. VDR-ablated animals fed this diet did not develop rickets or osteomalacia. However, alopecia was still observed in the VDR-ablated mice with normal mineral ions, suggesting that the VDR is required for normal hair growth. This study demonstrates that normalization of mineral ion homeostasis can prevent the development of hyperparathyroidism, osteomalacia, and rickets in the absence of the genomic actions of 1,25-dihydroxyvitamin D_3. Van Cromphaut et al[12,13] have also generated mice with deletions of the VDR and showed that the secondary hyperparathyroidism of these VDR-KO mice could be corrected by a high calcium diet.

Vitamin D may also amplify its effect on the parathyroid by increasing the activity of the calcium receptor (CaR). Canaff et al showed that in fact there are VDREs in the human CaR's promoter[14]. The calcium-sensing receptor (CaR), expressed in parathyroid chief cells, thyroid C-cells, and cells of the kidney tubule, is essential for maintenance of calcium homeostasis. They showed that parathyroid, thyroid, and kidney CaR mRNA levels increased 2-fold 15 h after intraperitoneal injection of $1,25(OH)_2D_3$ in rats. Vitamin D response elements (VDREs), in which half-sites (6 bp) are separated by three nucleotides, were identified in both promoters and shown to confer $1,25(OH)_2D_3$ responsiveness to a heterologous promoter. This responsiveness was lost when the VDREs were mutated. In summary, functional VDREs have been identified in the CaR gene and provide the mechanism whereby $1,25(OH)_2D_3$ up-regulates parathyroid CaR expression.

CALRETICULIN AND THE ACTION OF $1,25(OH)_2D_3$ ON THE PTH GENE

Another level at which $1,25(OH)_2D_3$ regulates PTH gene expression involves calreticulin. Calreticulin is a calcium binding protein which is present in the endoplasmic reticulum of the cell, and also may have a nuclear function. It regulates gene transcription via its ability to bind a protein motif in the DNA-binding domain of sterol nuclear hormone receptors. It has been shown to prevent vitamin D's binding and action on the osteocalcin gene *in vitro*[15]. Sela-Brown *et al*. showed that calreticulin might inhibit vitamin D's action on the PTH gene[16]. DNA binding studies and functional assays showed that calreticulin inhibited binding of the VDR-RXR complex to the vitamin D responsive element (VDRE) inhibited the effect of $1,25(OH)_2D_3$ on the PTH promoters. In addition, hypocalcemic rats had increased levels of calreticulin protein, as measured by Western blots, in their parathyroid nuclear faction. This may help explain why hypocalcemia leads to increased PTH gene expression despite high serum $1,25(OH)_2D_3$ levels, and may also be relevant to the refractoriness of the secondary hyperparathyroidism of many chronic renal failure patients to $1,25(OH)_2D_3$ treatment[16].

REGULATION OF PARATHYROID HORMONE GENE EXPRESSION BY SERUM CALCIUM AND PHOSPHATE

In uremic secondary hyperparathyroidism, PTH mRNA levels are increased[17]. Research into the regulation of PTH gene expression is significant to understanding the pathogenesis of secondary hyperparathyroidism. With limited amounts of preformed, mature PTH and rapid degradation of the hormone, much of the regulatory control of PTH occurs at the level of gene expression[18].

Like $1,25(OH)\text{-}_2D_3$, Ca^{++} and P determine the levels of PTH mRNA. Low serum Ca^{++} and high serum P increase PTH mRNA levels in the rat, and dietary manipulation of these minerals is sufficient to trigger the change[19,20]. However, unlike $1,25(OH)\text{-}_2D_3$, the regulatory actions of serum Ca^{++} and P are post-transcriptional[19,20]. Investigation of these post-transcriptional mechanisms has proved particularly interesting.

MECHANISM FOR POSTTRANSCRIPTIONAL REGULATION OF PARATHYROID HORMONE GENE EXPRESSION BY SERUM CALCIUM AND PHOSPHATE

Calcium and phosphate regulate parathyroid hormone (PTH) gene expression post-transcriptionally through the binding of *trans* acting factors to a defined *cis* acting instability element in the PTH mRNA 3'-untranslated region (UTR)[19,20]. We have defined AUF1 as a member of the protein-binding complex that protects PTH mRNA from degradation[22]. In hypocalcemia there is increased binding of the AUF1 containing complex to the PTH mRNA 3'-UTR that stabilizes the PTH mRNA leading to increased PTH mRNA and serum levels. Hypophosphatemia leads to the opposite effects[19]. It was further shown that a 63 nt element located in the PTH mRNA 3'-UTR was the determinant of PTH mRNA stability, since this element was sufficient to confer regulation by calcium and phosphate of PT proteins to a reporter gene in an in vitro degradation assay[23]. The 63 nt element also acts as an instability element in reporter genes in transfected cells in culture. The 63 nt element is AU rich but does not contain the classical AUUUA pentamer which defines it as a type III

ARE. Structure analysis of the PTH mRNA showed that the 3'-UTR and in particular the 63 nt element is dominated by significant open regions with little folded base pairing[24]. Therefore, the PTH mRNA 3'-UTR *cis* acting element is an open region that utilizes a distinct sequence pattern to determine mRNA stability by its interaction with *trans* acting factors.

A different pattern of effects on binding and stability occurs in a rodent model of chronic renal failure (rats with 5/6 nephrectomy). Cytosolic proteins from 5/6 nephrectomized rats do not show differences in PTH mRNA binding relative to control cytosolic proteins, but they stabilize PTH mRNA[17]. These findings suggest that the increased levels of PTH mRNA during chronic renal failure are due primarily to a reduction in cytosolic ribonuclease activity, resulting in a more stable PTH transcript.

Thus, the effects of hypocalcemia, hyperphosphatemia, and chronic renal failure on PTH gene expression all involve actions at the level of PTH mRNA stability, and more precisely, PT transcript protective and degrading proteins. Hypocalcemia and hypophosphatemia affect the binding of cytosolic protective factors and consequently PTH mRNA stability. Hypocalcemia stabilizes PTH mRNA by increasing the binding of cytosolic protective factors, whereas hypophosphatemia has the opposite effect. In rats with chronic renal failure, there is greater PTH mRNA stability by virtue of a decrease in the activity of degrading factors.

PT CELL PROLIFERATION

Parathyroid (PT) cells divide infrequently[25]. However, the PT cell retains the latent ability to proliferate into large hyperfunctioning glands in a number of clinical conditions. A common situation is that of the secondary hyperparathyroidism in most patients with CKD. Persistently low serum Ca^{++} or high serum P levels are the major factors leading to PT cell proliferation[26]. 1,25(OH)-vitamin D therapy directly decreases PTH gene transcription and PT cell proliferation [27,28]. However, vitamin D deficiency alone may cause the PT cell to proliferate because of the secondary chronic hypocalcemia[11].

Calcium is the major regulator of the PT, at the levels of secretion, gene expression as well as cell proliferation. In vivo, hypocalcemia leads to a profound increase in PT cell proliferation[26].

Studies of rats with chronic renal failure due to 5/6 nephrectomy reveal sensitive regulation of PT cell proliferation by serum P levels[26]. In rodents, a low P diet has been shown to prevent the five-fold increase in PT cell proliferation associated with chronic renal failure. This regulation by P is bidirectional; a high P diet increases PT cell proliferation to levels that are significantly greater than those in normally fed rats with experimental uremia.

Consistent with the patterns of regulation of PTH secretion and PTH gene expression, serum Ca^{++} regulates PT cell proliferation in a fashion opposite to serum P. A low calcium diet markedly increases PT cell proliferation[26] and in rats with chronic renal failure, administration of a calcimimetic compound that binds to the CaR attenuates the increase in PT cell proliferation[29,30].

Other research has begun to identify additional regulators of PT cell proliferation. These regulators include the endothelin receptor and the epidermal growth factor receptor. Antagonism of these signaling pathways decreases PT cell proliferation in rodent models of renal failure[28,31]. Collectively, these findings suggest several potential targets for the prevention of PT hyperplasia of secondary hyperparathyroidism, including Ca^{++}, P, endothelin, and epidermal growth factor signaling.

In patients with both primary and nodular secondary hyperparathyroidism due to chronic renal failure there is a decrease in VDR mRNA and protein levels[32-34]. In hyperparathyroidism there is a decrease in the cyclin kinase inhibitors p21 and p27 with an increase in TGFα in the parathyroids[28,34,35]. Treatment with vitamin D metabolites increases p21 levels and prevents the decrease in TGFα levels and prevent the parathyroid cell proliferation.

VITAMIN D METABOLITES IN THE MANAGEMENT OF PATIENTS WITH SECONDARY HYPERPARATHYROIDISM

Calcitriol and its analogs have an important place in the management of patients with CKD. Once vitamin D deficiency has been excluded by the measurement of serum 25(OH)-vitamin D levels then the serum calcium and phosphate should be maintained within the range defined in the KDOQI guidelines. This entails the judicious prescription of calcium containing phosphate binders within the recommended range so as not to provide too large a calcium dose. Serum PTH levels should now be maintained at about 2.5-3.0 fold the normal levels. In the majority of patients the serum PTH is too high and there are two therapeutic tools available, vitamin D metabolites and calcimimetic drugs. Calcitriol or its analogs can only be prescribed when the serum phosphate is within the target range and on condition that the serum calcium is not elevated. This can be achieved in most patients. There is no physiological advantage to prescribing parenteral calcitriol or its analogs apart from the tenuous claim of better patient compliance or the complexities of health insurance reimbursement arrangements. Calcitriol or analogs that are converted after absorption to $1,25(OH)_2$-vitamin D are the mainstay of treatment in many countries. With due attention to maintaining the serum calcium and phosphate within KDOQI guidelines this therapeutic strategy is successful in a large percentage of patients. However, there are many patients where there is an elevated phosphate and PTH despite this treatment and then a calcimimetic drug is indicated. Calcimimetics are rapidly becoming a cornerstone of treatment for patients with the secondary hyperparathyroidism of CKD.

Of particular interest are the recent reports of less morbidity in dialysis patients given calcitriol analogs as compared to calcitriol itself. These studies were retrospective and compared large numbers of dialysis patients treated with parenteral calcitriol as the historical controls to parenteral paricalcitol. They showed a reduction in hospitalizations and fewer episodes of hypercalemia as well as a decrease in mortality. These are potentially very exciting results. However, these were all retrospective studies and as the authors of the articles state 'prospective, randomized studies are critical to confirm these findings. The nephrology community eagerly awaits the outcomes of such trials and until then the reports should be treated with circumspection. Even more so, we await the results of studies showing the combined treatment with calcimimetics and vitamin D metabolites in patients with optimal control of serum phosphate. Our patients hopefully will have a marked improvement in their quality of life and life expectancy, but time will tell.

CONCLUSION

PTH gene expression is powerfully regulated by $1,25(OH)_2D_3$. This is a transcriptional effect and results in a marked decrease in PTH secretion and serum PTH. The effect of $1,25(OH)_2D_3$ on the parathyroid is used in the

treatment of many patients in chronic renal failure to prevent their secondary hyperparathyroidism. In addition, the expression of the PTH gene is also regulated by calcium and phosphate. These effects are post-transcriptional. The management of patients with CKD who have elevated serum PTH levels and are not vitamin D deficient by the judicious combination of oral phosphate binders, calcitriol or its analogs and calcimimetics should effectively maintain serum calcium, phosphate and PTH within the KDOQI guidelines. This will prevent the disabling complications of renal osteodystrophy and other systemic complications related to disordered mineral metabolism in these patients. The subset of patients with low turnover or adynamic bone disease is often associated with depressed serum PTH levels and may be a complication of over zealous use of calcitriol or its analogues together with calcium salts.

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