The role of calcium, phosphorus and vitamin D metabolism in the development of secondary hyperparathyroidism

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**Introduction**

Secondary hyperparathyroidism is a universal complication in patients with chronic renal failure. Abnormal calcium, phosphorus and vitamin D metabolism in chronic renal failure play a key role in the development of secondary hyperparathyroidism and renal osteodystrophy.

**The role of calcium**

The major factor involved in the regulation of parathyroid hormone (PTH) secretion is the concentration of ionized calcium in the extracellular fluid. A variety of experimental studies *in vivo* have demonstrated an inverse correlation between the extracellular calcium ion concentrations and hormone secretion. However, it is important to note that hormone secretion is not completely suppressed during hypercalcemia, and a basal rate of hormone secretion persists. The initial secretory response to hypo- or hypercalcemia occurs within seconds, suggesting that calcium acts directly on the plasma membrane. The mechanism of this effect may relate, at least in part, to an effect of calcium on parathyroid cell membrane potential. An additional effect of calcium on PTH secretion may also be a consequence of regulation of the amount of hormone available for secretion as a result of calcium-dependent regulation of hormone degradation within the parathyroid gland. A further effect of calcium may relate to the effects of the cation on membrane-bound adenylate cyclase activity and cyclic AMP accumulation (*vide infra*). The second response occurs when newly synthesized PTH is available for secretion after a prolonged decrease in extracellular ionized calcium (ICA) (several hours). The third response is the increase in parathyroid cell growth and number in response to hypocalcemia, resulting in elevation in PTH secretion in several days.

Brown *et al.* [1] cloned a calcium receptor localized in bovine parathyroid cell membranes. Although the molecular nature of such receptor(s) is unknown, parathyroid cells possess an extracellular calcium-sensing mechanism that recognizes trivalent and polyvalent cations (such as neomycin) and regulates PTH secretion by changes in phosphoinositide turnover and cytosolic calcium. This receptor features a large, extracellular domain containing clusters of acidic amino acids that are possibly involved in calcium binding. The extracellular domain is coupled to a cellular membrane-spanning domain similar to the G-protein-coupled receptor superfamily. Pollack *et al.* [2] demonstrated that mutations in the human calcium-sensing receptor cause familiar hypocalciuric hypercalcaemia and severe neonatal hyperparathyroidism.

Regulation of the expression of the calcium sensor could have major physiological and pathological implications. Studies *in vivo* in the rat parathyroid glands and kidney [3,4] and *in vitro* in cultured bovine parathyroid cells [5] have indicated that this receptor is not regulated by extracellular calcium. Vitamin D deficiency has been shown to decrease and 1,25-dihydroxy vitamin D₃ [1,25-(OH)₂D₃] to increase calcium sensor mRNA in the rat [4], suggesting an additional level of control by 1,25-(OH)₂D₃, but this remains controversial [3].

Calcium has also been demonstrated to control PTH mRNA levels *in vivo* and *in vitro*. In the rat, Naveh-Many *et al.* [6] showed that hypocalcaemia increased PTH mRNA, whereas hypercalcaemia had no effect. The effect of low calcium was confirmed by Yamamoto *et al.* [7], but this group observed a decrease in PTH mRNA in hypercalcaemic rats. Okazaki *et al.* [8] identified a negative regulatory element located 3.5 kb upstream from the transcriptional start site in the PTH gene that modulates the transcriptional suppression by extracellular calcium when fused to a chloramphenicol acetyltransferase (CAT) reporter and tested in baby hamster kidney (BHK) cells. This motif consists of
putative palindromic structure formed from two six-base elements separated by 3 bp and is similar to the negative calcium-responsive elements in the atrial natriuretic peptide and renin genes. The relevance of the calcium response element in the PTH gene will require confirmation in a parathyroid cell line. While considerable information has been obtained regarding the control of PTH secretion from normal parathyroid tissue, the control of PTH secretion by abnormal parathyroid glands is ill defined. The observations that elevated calcium concentrations do not completely suppress PTH secretion from normal parathyroid tissue and that hypercalcemia can be induced by transplantation of multiple normal parathyroids suggest that the persistent basal secretion of PTH may be of physiological significance when parathyroid mass is increased. This phenomenon may contribute to the apparent non-suppressibility of PTH secretion in hyperparathyroidism. Alternatively, it is also possible that there is an intrinsic abnormality in the response to calcium of abnormal parathyroid tissue, as has been demonstrated in parathyroid tissue from patients with primary hyperparathyroidism. Since parathyroid hyperplasia is a universal phenomenon in chronic renal failure, it is important to consider the possibility that the response of parathyroid tissue to calcium from patients with chronic renal failure might also be abnormal. Bellorin-Font et al. [9] demonstrated that parathyroid adenylate cyclase kinetics were abnormal in hyperplastic parathyroid glands from patients with chronic renal failure and similar to that seen in parathyroid adenoma. The adenylate cyclase of pathological parathyroid tissue had an increased affinity for magnesium and a reduced sensitivity to inhibition by calcium. The altered affinity for magnesium could be corrected by guanosine triphosphate in vitro, suggesting the possibility that the abnormal regulation of adenylate cyclase was at or closely related to the guanyl nucleotide regulatory site of adenylate cyclase. Brown et al. demonstrated abnormal calcium-regulated PTH release in isolated parathyroid cells from uremic patients [10]. These hyperplastic glands demonstrated an increased 'set-point' for calcium suppression of PTH release.

Altered feedback regulation of PTH by calcium in renal failure

Hyperplastic parathyroid glands display less sensitivity to calcium than does normal tissue [10]. These observations suggest that a mechanism for increased PTH levels in chronic renal failure may be a shift in the set-point for calcium-regulated PTH secretion as well as an increase in parathyroid tissue mass. The shift in the set-point for calcium-regulated PTH secretion is also manifested as an increase in the calcium concentration required for inhibition of adenylate cyclase activity in membranes prepared from hyperplastic parathyroid glands obtained from patients with chronic renal insufficiency [9]. Not only is the set-point for calcium-regulated hormone secretion elevated in cells from hyperplastic parathyroid tissue, but also the degree of responsiveness across the calcium-sensitive range is altered.

Several factors can thus lead to elevated serum parathyroid hormone levels in man. These include: (i) an increase in tissue mass either from cell hypertrophy and/or hyperplasia; (ii) an increase in the set-point for calcium to inhibit hormone secretion; and (iii) a change in the degree of suppression by calcium throughout the calcium-sensitive range (e.g. slope of the suppression line). Kifor et al. [11], using immunohistochemistry techniques, demonstrated a 59% decrease in calcium-sensing receptor (CaR) in parathyroid glands of uraemic patients. Similar results were demonstrated in adenomas of parathyroid glands obtained from patients with primary hyperparathyroidism. These investigators concluded that the degree of CaR reduction would be sufficient to account, at least in part, for the altered sensitivity to calcium observed in secondary hyperparathyroidism.

The role of phosphate retention

Several investigators have demonstrated that phosphorus retention plays an important role in the genesis of secondary hyperparathyroidism. The mechanisms by which this effect occurs are complex and somewhat controversial. The mechanisms considered are as follows: (i) phosphorus-induced decrease in 1,25-(OH)₂D₃ levels; (ii) phosphorus-induced hypocalcaemia; and (iii) phosphorus-induced hyperparathyroidism, independent of changes in ICa and 1,25-(OH)₂D₃. However, it is important to emphasize that these mechanisms are closely interrelated and are not mutually exclusive. Since phosphorus regulates the production of 1,25-(OH)₂D₃ by altering the renal enzyme, 25-hydroxyvitamin D-1α-hydroxylase, it is possible that the effect of phosphorus retention is mediated by a decrease in the synthesis of 1,25-(OH)₂D₃. Conversely, a beneficial effect of phosphorus restriction in ameliorating hyperparathyroidism could be explained by increased levels of 1,25-(OH)₂D₃. Portale et al. [12] demonstrated that, in patients with moderate renal insufficiency, phosphate restriction increased plasma 1,25-(OH)₂D₃ with a concomitant normalization of plasma PTH. This occurred despite no change in serum phosphorus. Thus, the effect of phosphorus on the 1α-hydroxylase in early renal insufficiency may not be responsible for the development of hypocalcaemia that may be seen in patients with advanced renal failure and severe hyperphosphataemia. Since reduced renal mass may limit the production of 1,25-(OH)₂D₃ in advanced renal insufficiency, we performed further studies [13] to clarify the mechanism by which dietary phosphate restriction improved secondary hyperparathyroidism. We examined in uraemic dogs how dietary phosphate restriction could ameliorate secondary hyperparathyroidism without increasing 1,25-(OH)₂D₃. Our results
confirmed an important effect of phosphorus restriction on suppressing secondary hyperparathyroidism. However, in contrast to previous findings in patients with moderate renal insufficiency, progressive reduction of dietary phosphorus from 0.9 to 0.3% did not increase plasma 1,25-(OH)$_3$D$_3$. Thus, in severe chronic renal failure, phosphorus appears to regulate PTH secretion by a mechanism that is independent of calci-triol. In agreement with our results, Lucas [14] found that, despite the administration of a low phosphorus diet to patients with advanced renal insufficiency, 1,25-(OH)$_2$D$_3$ did not increase. Plasma PTH significantly decreased and no change in serum calcium was observed. Schaefer [15] obtained similar results in a group of 17 patients with advanced renal insufficiency (plasma creatinine 8.5 mg/dl) whose diet contained ketoacids. After 8 weeks of treatment, they found a significant decrease in plasma phosphorus and intact PTH (iPTH). There were no changes in plasma calcium, 1,25-(OH)$_2$D$_3$ or 25-(OH)$_2$D$_3$. In vitamin D-deficient rats, Dabbagh et al. [16] observed that the administration of a phosphorus-restricted diet prevented the development of secondary hyperparathyroidism. Thus, in the absence or extreme low levels of 1,25-(OH)$_2$D$_3$ and slightly reduced serum calcium, a low phosphorus diet prevents the development of hyperparathyroidism.

Recently we have shown that dietary phosphate restriction prevented hyperplasia in uraemic rats [17]. Studies were performed in normal and uraemic rats fed a low (0.2%) or high phosphorus (0.8%) diet for a period of 2 months. Parathyroid gland weight and serum PTH were similar in both groups of normal rats and uraemic rats fed the 0.2% phosphorus diet. On the other hand, in uraemic rats fed the 0.8% phosphorus diet, the parathyroid glands weight increased by ~120% compared with the normal animals fed the same diet. In the uraemic rats fed the high phosphorus diet, PTH increased from 29.1 ± 6.1 to 130 ± 25 pg/ml. There were no changes in ICa or 1,25-(OH)$_2$D$_3$. In vitro studies with parathyroid glands of normal rats demonstrated that, when the phosphorus in the culture medium was increased from 0.2 to 2.8 mM, the amount of PTH secreted into the medium increased from 810 ± 155 to 1492 ± 182 pg/µg DNA/5 h (Figure 1). Since it took a minimum of 3 h for phosphorus to increase the amount of PTH in the medium, the effect of phosphorus was mainly on PTH synthesis and eventually on secretion. The addition of cycloheximide blocked the effect of phosphorus, suggesting that protein synthesis is necessary for the increment in PTH secretion. Other investigators also have clearly demonstrated a significant effect of phosphorus on PTH secretion and parathyroid cell proliferation [18–21].

In conclusion, dietary phosphorus restriction improves secondary hyperparathyroidism in animals and subjects with advanced renal failure. This effect is not mediated by an increase in 1,25-(OH)$_2$D$_3$ or plasma ICa. In addition, high phosphorus diet induces chief cell hyperplasia of parathyroid glands in uraemic rats. Moreover, studies in vitro demonstrate that high phosphorus levels increase PTH synthesis and secretion post-transcriptionally. Thus, in addition to a well-known effect of phosphorus in the regulation of 1,25-(OH)$_2$D$_3$ synthesis, a high phosphorus diet may have a direct effect on the secretion of PTH. Although the mechanism of this effect is not yet known, phosphorus potentially may affect the phospholipid composition of the parathyroid cell membrane, calcium fluxes and vitamin D receptors and perhaps have an effect on the calcium receptor in the parathyroid cell membrane. From the clinical point of view, significant data have accumulated indicating that, in the presence of hyperphosphataemia, the parathyroid glands are resistant to the action of 1,25-(OH)$_2$D$_3$. The new evidence for a direct action of phosphorus on PTH synthesis and chief cell hyperplasia emphasizes the importance of controlling serum phosphorus in chronic renal failure. Further studies are necessary to determine the precise mechanism at the molecular level by which phosphorus contributes to the regulation of PTH secretion in chronic renal failure.

**The role of vitamin D**

In renal failure, a series of alterations characterized by abnormal production, altered metabolism, decreased number of vitamin D receptors and resistance to the action of vitamin D are of utmost importance in the pathophysiology of secondary hyperparathyroidism. Nearly all of the action of 1,25-(OH)$_2$D$_3$ are mediated by the intracellular vitamin D receptors. Korkor [22] demonstrated that 1,25-(OH)$_2$D$_3$ binding activity in...
parathyroid gland from chronic renal failure patients was lower than in parathyroid adenomas or parathyroid glands from transplant recipients. The decrease was attributed to a decrease in maximal binding, with no change in affinity for 1,25-(OH)$_2$D$_3$. Fukuda et al. [23] performed immunohistochemistry on parathyroid tissue from patients with chronic renal failure, and found a greater decrement in vitamin D receptor staining in the nodular portions than in the diffuse hyperplastic region of the parathyroid glands. Decreased parathyroid gland vitamin D receptor content has been confirmed in several subsequent studies using uremic rats [24] and dogs [25]. Szabo et al. [26] suggested that the decreased vitamin D receptor content in the parathyroid glands of uremic rats was due to ex vivo degradation. When protease inhibitors were included during tissue preparation, vitamin D receptor levels were found to be greater in uremic glands. However, recently, Denda et al. [27] reported a lower vitamin D receptor content in the parathyroid glands of uremic rats despite the use of protease inhibitors. The reason for these disparate results in uremic rat models are unclear. Examination of the serum chemistries in the two studies reveals differences in serum phosphorus. In the studies by Szabo et al. [26], the uremic rats had lower serum phosphorus than the normal controls, whereas uremic rats in the study by Denda had higher serum phosphorus. Down-regulation of the vitamin D receptors in parathyroid glands in uremic rats appear to be post-transcriptional. Denda showed a strong correlation between serum 1,25-(OH)$_2$D$_3$ and vitamin D receptor binding activity in the parathyroid glands of uremic rats, suggesting that the decreased vitamin D receptors may be related to the low levels of 1,25-(OH)$_2$D$_3$. Low doses of 1,25-(OH)$_2$D$_3$ (2 or 6 ng, three times per week) or the administration of 22-oxacalcitriol increased the vitamin D receptor to normal levels (Figure 2). Since there was no regulation of a vitamin D receptor mRNA by these low doses of 1,25-(OH)$_2$D$_3$ (unpublished data), in agreement with Shvil et al. [28] the regulation of vitamin D receptor binding activity by 1,25-(OH)$_2$D$_3$ appears to be due to ligand-dependent stabilization of the vitamin D receptor protein.

Resistance to 1,25-(OH)$_2$D$_3$ in chronic renal failure also appears to occur at the level of vitamin D receptor activity. Patel and colleagues [29] have presented evidence that components in the blood of renal failure patients diminished the ability of the vitamin D receptor to bind to DNA. Intestinal vitamin D receptor from uremic rats was found to elute from DNA cellulose at a lower salt concentration, indicating reduced affinity for DNA. Nuclear uptake studies performed in vitro showed the intestinal vitamin D receptor from uremic rats was taken up to lesser extent than the vitamin D receptor from normal rat intestine [30]. Incubation of normal vitamin D receptor with uremic plasma ultrafiltrate inhibited nuclear uptake in vitro. Patel et al. [31] determined the effect of uremic plasma ultrafiltrate on binding of vitamin D receptor to a known vitamin D responsive element (VDRE), using an electrophoretic mobility shift assay (EMSA). Analysis of the data indicates that an ultrafiltrate did not effect the affinity of the binding but rather reduced the fraction of vitamin D receptor capable of binding the VDRE. The question of whether this in vitro effect of uremic plasma ultrafiltrate could alter vitamin D receptor activity in vivo was answered in part by the demonstration that this plasma fraction could inhibit the 1,25-(OH)$_2$D$_3$ mediator transcriptional activation of a reporter gene in a whole cell model. The component present in the blood of renal failure patients that inhibits vitamin D receptor action has not been identified completely. The primary role of 1,25-(OH)$_2$D$_3$ in controlling PTH synthesis is at a transcriptional level [32]. Recent studies have identified VDREs in the human and chicken PTH genes [33,34]. The elements from the two species appear to differ markedly in their structure and binding properties. These studies have been hampered by the lack of a parathyroid cell line, and many questions remain as to the mechanisms of transcriptional suppression of the PTH gene. It is clear though that the effect is mediated by the nuclear vitamin D receptor. Thus, the low serum 1,25-(OH)$_2$D$_3$ and the decreased vitamin D receptor content in the parathyroid glands in renal failure will lead to the overexpression of the PTH gene. Calcitriol has been shown to up-regulate the vitamin D receptor binding and the receptor mRNA (high doses of 1,25-(OH)$_2$D$_3$ [35] in the parathyroid glands of experimental animals. As discussed above, the lower levels of serum 1,25-(OH)$_2$D$_3$ in chronic failure may be the critical factor in the down-regulated vitamin D receptor expression of serum parathyroid glands in this condition.

1,25-(OH)$_2$D$_3$ may also be critical in the full responsiveness of the parathyroid gland to calcium.
Heterologous regulation of the calcium-sensing receptor of the rat parathyroid glands by 1,25-(OH)2D3 have been reported [4]. Vitamin D deficiency decreased and 1,25-(OH)2D3 treatment increased the calcium-sensing receptor mRNA. Clinical observations suggest that replacement therapy with 1,25-(OH)2D3 to control PTH is difficult when chronic renal failure patients have hyperphosphataemia [36]. The nature of this resistance to 1,25-(OH)2D3 is unclear and may involve several alterations in parathyroid glands such as vitamin D receptor calcium receptor expression or activation in these patients.

In summary, in chronic renal failure patients the parathyroid gland become hyperplastic. This is due, at least in part, to chronic hypocalcaemia, which may involve vitamin D deficiency and resistance in intestine and bone as discussed above. In addition, hyperphosphataemia also may play a critical role in the development of chief cell hyperplasia. There is evidence that 1,25-(OH)2D3 can regulate parathyroid cell proliferation. In vitro studies have shown that 1,25-(OH)2D3 can inhibit the serum-stimulated proliferation of parathyroid cells. Vitamin D deficiency leads to parathyroid hyperplasia, but this may be due in part to the concomitant hypocalcaemia. Undoubtedly, the reduced number of vitamin D and calcium-sensing receptors also may play an important role in the development of secondary hyperparathyroidism.

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