Pathogenesis of secondary hyperparathyroidism

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Abstract. Hyperplasia of the parathyroid glands and increased concentrations of immunoreactive parathyroid hormone are among the earlier alterations of mineral metabolism in patients with chronic renal failure. In the past five years several investigators have demonstrated that phosphorus retention plays a key role in the development of secondary hyperparathyroidism and chief cell hyperplasia of the parathyroid glands. Since phosphorus regulates the production of 1,25D3 by altering the enzyme 1α-hydroxylase it is possible that the effect of phosphorus retention is mediated by a decrease in the synthesis of 1,25D3. This has been shown in patients with early renal insufficiency. However, in patients with advanced renal failure the reduced renal mass may limit the production of 1,25D3. It is clear now that phosphorus per se independent of the levels of ionized calcium and 1,25D3 can increase the synthesis and secretion of PTH in vivo and in vitro. The abnormalities in vitamin D metabolism are not only characterized by low levels 1,25D3 but by low number of vitamin D receptors. Thus, the parathyroid glands are resistant to the action of 1,25D3 and high pharmacological concentrations of 1,25D3 in blood are necessary to suppress the levels of parathyroid hormone in advanced renal failure. The development of monoclonal changes in glands obtained from patients with secondary hyperparathyroidism further complicates the treatment of secondary hyperparathyroidism in patients maintained on hemodialysis. Thus, correction of serum phosphorus is imperative for the success of 1,25D3 to control the levels of parathyroid hormone. Currently several laboratories are studying at the molecular level the mechanisms by which dietary phosphorus induces chief cell hyperplasia.

Key words: calcitriol; calcium; secondary hyperparathyroidism; phosphorus; uraemia

Introduction

The kidneys play a key role in the metabolism of parathyroid hormone (PTH) and vitamin D. Hyperplasia of the parathyroid glands and increased concentrations of immunoreactive PTH are among the earlier alterations of mineral metabolism in patients with chronic renal failure. The two major factors responsible for the development of secondary hyperparathyroidism are phosphorus retention and diminished 1,25(OH)2D3 (1,25D calcitriol). It is well known that in health, ionized calcium (ICa) and 1,25D are the two major regulators of PTH homeostasis. The interplay in the regulation of PTH secretion may not be the same in uraemia. The action of ICa on PTH secretion is very fast (<3 min) and that of 1,25D is slow (>6 h). Thus, 1,25D plays a key role in the day-to-day maintenance of calcium balance but its acute effects are of little significance. PTH stimulates the production of 1,25D by activating the 1α-hydroxylase enzyme, and 1,25D suppresses the synthesis of PTH. Thus, both PTH and 1,25D directly affect calcium homeostasis and each exercises important regulatory effects on the other.

1,25D and calcium are negative regulatory elements in the 5’-upstream region of the PTH gene. The calcium responsive element was found around −3.5 kb upstream from the human PTH gene, and its sequence consists of 12 palindromic bases (TGAGAC) with a gap of three bases (AGG) [1]. In contrast the negative vitamin D responsive element was found in 25 base pair oligonucleotides from −125 to −101 [2]. In order to determine the relationship of these two factors (ICa and 1,25D) in the suppression of PTH in uraemic rats, we induced hypocalcaemia by feeding uraemic rats a diet deficient in calcium. We demonstrated that pharmacological doses of 1,25D that usually suppress secondary hyperparathyroidism failed to suppress both the synthesis of pre-pro PTH mRNA and PTH secretion. Thus, correction of both calcium and 1,25D is crucial in the treatment of secondary hyperparathyroidism.

Alterations in vitamin D metabolism in renal failure

The control of PTH gene transcription by 1,25D is thought to be mediated by a receptor protein in target cells that has a high affinity and high specificity for the vitamin D metabolite. Although the mechanisms...
by which the receptor carries out the nuclear action of 1,25D are not fully understood, it is clear that the receptor can determine the response of the target cell to 1,25D. Korkor [3] demonstrated that parathyroid glands taken from patients with chronic renal failure (CRF) contained one-third the number of receptors compared to parathyroid adenomas. Merke et al. [4] found that the parathyroid glands of uremic rats contained only half the number of receptors compared to parathyroid glands of sham-operated controls. Similar results were found by Brown et al. [5] in dogs. Although it has not been rigorously proven that the vitamin D receptor (VDR) plays a role in suppressing PTH synthesis and determining the set-point for calcium, it is possible that the reduced VDR numbers in the parathyroid glands of uremic patients render the glands less responsive to the inhibitory action of 1,25D. Whether serum 1,25D determines the content of VDR in parathyroid glands is unclear. Naveh-Many et al. [6] demonstrated that the administration of 1,25D led to a dose-dependent increase in the mRNA for the VDR in the parathyroid glands of normal rats. These data are consistent with the view that 1,25D up-regulates its own receptor in parathyroid cells. An additional effect of 1,25D on parathyroid glands comes from the studies of Kremer et al. [7], who showed that exposure of quiescent, cultured bovine parathyroid cells to serum resulted in increased \[^{3}H\] tyramine incorporation, followed by an increase in the cell number. These changes were preceded by an increase in c-myc and c-fos proto-oncogene mRNA levels. However, 1,25D, when added to culture medium, blocked the increase in c-myc mRNA. There was no increase in the number of parathyroid cells. These results indicate that 1,25D may directly modulate parathyroid cell proliferation by altering the expression of replication associated with specific proto-oncogenes. Studies by Szabo et al. [8] in rats with renal failure suggest that 1,25D administration suppresses parathyroid hyperplasia independent of changes in serum calcium. Once established, hyperplasia was not reversed by short-term 1,25D treatment.

Fukuda et al. [9] provided evidence for a decreased 1,25D receptor density in patients with severe parathyroid hyperplasia. The investigators studied the VDR distribution of surgically excised parathyroid glands obtained from dialysis patients. They classified the parathyroid glands as exhibiting nodular or diffuse hyperplasia. They found a lower density of the VDR in parathyroid glands showing nodular compared to diffuse hyperplasia. A significant negative correlation was found between VDR density and the weight of the parathyroid gland. In other words, the greater the serum PTH, the greater the degree of parathyroid gland hyperplasia and the lower the density of the VDR. These studies provide a rational basis for understanding the difficulties in suppressing secondary hyperparathyroidism when PTH is extremely high (>1500 pg/ml). In addition, it is important to emphasize that there is a component of the secretory mechanism in parathyroid glands that cannot be suppressed by either high ICa or pharmacological doses of 1,25D. Although this non-suppressible component represents a small percentage of the total amount of PTH secreted by the parathyroid glands in normal individuals, it becomes extremely important in those patients in which the parathyroid glands size is 50–100 times greater than normal. Gittes et al. [10] demonstrated this phenomenon by implanting a large number of parathyroid glands into rats having undergone a previous parathyroidectomy. The serum calcium decreased after parathyroidectomy. However, after the implantation of 20 or 80 parathyroid glands, the rats developed severe hypercalcaemia. One would expect that the hypercalcaemia should suppress the release of PTH. It was clear from these experiments that the hypercalcaemia continued because a non-suppressible component was present, and the large amount of tissue was responsible for the maintenance of hypercalcaemia. Furthermore, when Mayer et al. [11] measured the A-V difference of PTH across the parathyroid glands in cows, they found that, despite severe hypercalcaemia (serum calcium up to 20 mg/dl), there still was a small component of PTH secretion. Thus, the development of hyperplasia with a consequent increase in the non-suppressible component plus the low number of receptors makes the parathyroid gland more resistant to the use of 1,25D in the treatment of severe secondary hyperparathyroidism.

Abnormal calcium set-point

Evidence exists for an intrinsic abnormality of the parathyroid glands in uremia that leads to disordered calcium-regulated PTH secretion. An insensitivity to the suppressive effects of calcium on PTH secretion has been shown in glands obtained from patients with CRF [12,13]. These observations suggest that one mechanism for the increased PTH in CRF may be a shift in the set-point for calcium-regulated PTH secretion in addition to the increase in the mass of parathyroid tissue. The set-point for calcium in normal parathyroid glands was approximately 1.0 mmol calcium, whereas in patients with secondary hyperparathyroidism, the set-point was found to be 1.26 mmol calcium. These abnormalities are also manifested by an increase in the calcium concentration required for the inhibition of the adenylate cyclase activity in membranes prepared from hyperplastic parathyroid glands obtained from patients with CRF [14]. It is possible, therefore, that normal concentrations of ICa in serum may not be sufficient to suppress PTH secretion in hyperplastic parathyroid glands. Thus, the serum calcium may have to be increased to the upper limits of normal to control the increase of PTH in patients with secondary hyperparathyroidism. Although the precise mechanism responsible for the decreased sensitivity to calcium in hyperplastic parathyroid glands is unknown, alterations in vitamin D metabolism (i.e. reduced 1,25D and decreased numbers of VDR in the para-
thyroid glands may account, in part, for the abnormal secretion of PTH in renal failure.

To further characterize the potential role of 1,25D on the abnormal set-point for the suppression of PTH by calcium, Delmez et al. [15] studied the suppression of PTH by calcium before and after 2 weeks of intravenous (IV) 1,25D in a group of haemodialysis patients. During hypercalcemic suppression, the calcium set-point for PTH declined from 5.24 to 5.06 mg/dl after the administration of 1,25D. During hypocalcemic stimulation, the parathyroid response was attenuated by 1,25D. Thus, the suppression of PTH secretion during treatment with 1,25D appears to be due, in part, to an increase in the sensitivity of the parathyroid glands to ambient calcium concentrations (Figure 1). Dunlay et al. [16] found similar inhibitory effects with IV 1,25D in dialysis patients. After 10 weeks of IV 1,25D, there was a significant decrease in serum PTH. These investigators also found that the ionized calcium–PTH sigmoidal curve was shifted to the left, suggesting an increase in the suppressive effect of calcium. Several years ago we demonstrated that the IV administration of 1,25D was very effective in the suppression of secondary hyperparathyroidism [17]. Studies performed in 20 patients maintained on chronic haemodialysis and given IV 1,25D demonstrated a marked suppression of PTH (70.1 ± 3.2%). On the other hand, studies performed in a group of patients treated with oral daily doses (0.5 ug) of 1,25D showed less suppression. We also demonstrated that the suppression of PTH by 1,25D was dose dependent in primary culture of bovine parathyroid cells. Tsukamoto et al. [18] showed that 1,25D, when given as an oral pulse of 3–4 ug twice weekly, instead of 0.5 ug daily, achieved a greater suppression.

Reichel et al. [19] compared the effects of intermittent versus continuous administration of 1,25D in uraemic rats. One group of animals received 35 pmol 1,25D intraperitoneal (IP) bolus on days 0 and 4. A second experimental group received a continuous infusion of 70 pmol 1,25D over 6 days via an osmotic mini-pump. Thus, over the same period of time, the two groups received the same amount of 1,25D. Peak calcitriol concentrations were significantly greater and PTH values significantly less in the group that received the two IP boluses compared to the group that received the continuous infusion (Figure 2). The degree of suppression of the pre-pro PTH/β-actin mRNA was also greater in the group that received the IP bolus versus those animals treated with a constant infusion. Moreover, the growth of the parathyroid glands was prevented only with the bolus administration (Figure 3). Thus, these investigators conclude that the concentration of 1,25D achieved in serum is an important determinant of the response of the parathyroid glands to 1,25D.

Brown et al. [20] cloned a calcium receptor (CaR) localized in bovine parathyroid cell membranes. Although the molecular nature of such receptor(s) is
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The role of phosphorus

Several investigators have demonstrated that phosphorus retention plays an important role in the genesis of secondary hyperparathyroidism. However, the mechanisms by which this effect occurs are complex and somewhat controversial. The mechanisms considered are: (1) phosphorus-induced decrease in 1,25D; (2) phosphorus-induced hypocalcaemia; and (3) phosphorus-induced hyperparathyroidism, independent of changes in ICa and 1,25D. It is important to emphasize that these mechanisms are closely interrelated and are not mutually exclusive. Since phosphorus regulates the production of 1,25D by altering the enzyme 1α-hydroxylase [24], it is possible that the effect of phosphorus retention is mediated by a decrease in the synthesis of 1,25D. Conversely, a beneficial effect of phosphorus restriction in ameliorating hyperparathyroidism could be explained by increased 1,25D. Portale et al. [25] demonstrated that, in patients with moderate renal insufficiency, phosphorus restriction increased plasma 1,25D with a concomitant normalization of plasma PTH. This occurred despite no change in serum phosphorus and calcium. 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 1,25D in advanced renal insufficiency, we performed further studies [26] in uraemic animals to clarify the mechanisms by which dietary phosphate restriction improved secondary hyperparathyroidism. We examined how dietary phosphate restriction could ameliorate secondary hyperparathyroidism without increasing 1,25D. 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 1,25D. Thus, in severe CRF, phosphorus appears to regulate PTH secretion by a mechanism that is independent of calcitriol. In agreement with our results, Lucas [27] found that, despite the administration of a low phosphorus diet to patients with advanced renal insufficiency, 1,25D did not increase. Plasma PTH, however, significantly decreased without changes in serum calcium concentrations. Schaefer [28] obtained similar results in a group of 17 patients with advanced renal insufficiency (plasma creatinine 8.5 mg/dl) in whom ketoacids were added to the diet. After 8 weeks of treatment, they found a significant decrease in plasma phosphorus and PTH. There were no changes in plasma calcium, 1,25D or 25(OH)D3. Tessitore et al. [29] also showed that dietary phosphorus restriction did not have an effect on 1,25D concentration when the GRF was less than 20 ml/min. In vitamin D-deficient rats, Dabbagh et al. [30] observed that, in the presence of normal plasma calcium concentrations, the administration of a phosphorus-restricted diet prevented the development of secondary hyperparathyroidism. Recently we have demonstrated that phosphate restriction prevented parathyroid cell growth in uraemic rats and high phosphate directly stimulated...
Pathogenesis of secondary hyperparathyroidism (modified from Fig. 5. Diagrammatic representation of factors involved in the diet) PTH also increased from 29.1 ± 6.1 to 810 ± 155 to 1492 ± 182 pg/ug DNA/5 h. Since it took a minimum of 3 h for phosphorus to increase the effect of phosphorus on PTH secretion in tissue culture [31]. Studies were performed in normal and uraemic rats fed a low phosphorus (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% P diet. On the other hand, in uraemic rats fed the 0.8% P diet, the parathyroid gland weight increased by approximately 120% compared to the normal animals fed the same diet. In this group of rats (fed a 0.8% P diet) PTH also increased from 29.1 ± 6.1 to 130 ± 25 pg/ml. There were no changes in ICA or 1,25D. Studies in vitro with parathyroid glands of normal rats demonstrated that, when the phosphorus in the culture medium was increased from 0.2 to 2.8 mM, PTH secreted into the medium increased from 810 ± 155 to 1492 ± 182 pg/µg DNA/5 h. Since it took a minimum of 3 h for phosphorus to increase the amount of PTH in the medium, the effect of phosphorus is mainly on PTH synthesis and eventually on secretion.

In conclusion, dietary phosphorus restriction improved secondary hyperparathyroidism in animals and subjects with advanced renal failure. This effect was not mediated by an increase in 1,25D or plasma ionized calcium. In addition, high phosphorus diets induced chief cell hyperplasia of parathyroid glands in uraemic rats. Moreover, studies in vitro demonstrated that phosphorus increases PTH synthesis and secretion. Thus, in addition to a well-known effect of phosphorus in the regulation of 1,25D, a high phosphorus diet may have direct effect on the secretion of PTH. Although the mechanism of this effect is not yet known, phosphorus may also potentially affect the phospholipid composition of the parathyroid cell membrane, calcium fluxes and VDR, 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 1,25D. The new information of a direct action of phosphorus on PTH synthesis and chief cell hyperplasia further 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 CRF.

An integrated scheme of the factors involved in the pathogenesis of secondary hyperparathyroidism is depicted in Figure 5.

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References


Fig. 5. Diagrammatic representation of factors involved in the pathogenesis of secondary hyperparathyroidism (modified from [32]).
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