New insights into the regulation of parathyroid hormone synthesis and secretion in chronic renal failure

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Abstract. The main factors which regulate parathyroid hormone (PTH) production are calcium, phosphate, vitamin D and the sex steroids, estrogens and progesterins. Hypocalcaemia leads to increased PTH secretion in seconds and minutes, gene expression in hours and parathyroid cell number in weeks and months. Hypercalcemia leads to a decrease in PTH secretion by its action on the parathyroid cell calcium receptor and no decrease in PTH mRNA concentrations. There is now convincing evidence that phosphate regulates the parathyroids independent of its effect on serum calcium and 1,25-dihydroxyvitamin D3 (1,25(OH)2D3). In vivo in rats hypophosphataemia markedly decreases PTH mRNA and serum PTH independent of its effect on serum calcium and 1,25(OH)2D3. Clinical studies also indicate that phosphate regulates the parathyroids independent of its effect on serum calcium and 1,25(OH)2D3. 1,25(OH)2D3 itself has a marked effect on the parathyroids where it decreases PTH gene transcription by a direct action. Parathyroid cell proliferation is regulated by dietary calcium and phosphate with hypocalcaemia markedly increasing and hypophosphataemia markedly decreasing the number of proliferating cells. The application of basic science findings of how calcium, phosphate and 1,25(OH)2D3 regulate the parathyroids has led to an efficient and safe prescription for the management of the secondary hyperparathyroidism of chronic renal failure which is the maintenance of a normal serum calcium and phosphate and the careful use of bolus doses of 1,25(OH)2D3.

Key words: calcium; cell proliferation; phosphate; parathyroid hormone; secondary hyperparathyroidism; vitamin D

Vitamin D and the parathyroid

The story of vitamin D and the parathyroid has been a success story for the application of discoveries in basic science to clinical medicine. Soon after 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) was discovered as the biologically active form of vitamin D by Fraser [1] it was used to treat patients with renal osteodystrophy. The initial resounding clinical successes were attributed to the effective treatment of the osteomalacia of renal osteodystrophy by the action of 1,25(OH)2D3 on bone. The discovery in vitro in primary cultures of bovine parathyroid cells that 1,25(OH)2D3 had a direct action to decrease parathyroid hormone (PTH) mRNA [2] introduced a further dimension to the action of 1,25(OH)2D3 and its use in patients with renal failure. This was substantiated by the in vivo findings of Silver et al. [3] that 1,25(OH)2D3 led to a dramatic decrease in PTH gene transcription in normal rats at physiologically relevant doses. 1,25(OH)2D3 acts by binding to its specific 1,25(OH)2D3 receptor in its target organs and Naveh-Many et al. [4] showed by in situ hybridization that the 1,25(OH)2D3 receptor mRNA was present in the parathyroid of rats and not in the surrounding thyroid. In addition they showed that the concentration of the receptor mRNA in the parathyroid was the same as that in the duodenum, the classical target organ for 1,25(OH)2D3. They therefore established that the parathyroid was a physiological target organ for 1,25(OH)2D3. Shvil et al. [7] showed that 1,25(OH)2D3 was as effective in rats with experimental uremia due to 5/6 nephrectomy. This was the experimental background for the use of 1,25(OH)2D3 as effective treatment for the secondary hyperparathyroidism of chronic renal failure. Since then there has been an accumulation of a large amount of clinical experience that 1,25(OH)2D3 is the basis of the prevention and treatment of the secondary hyperparathyroidism of uraemia on condition that the serum phosphate is not allowed to increase excessively.
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Because of the potential of 1,25(OH)\(_2\)D\(_3\) to increase serum calcium there has been much interest in analogues of 1,25(OH)\(_2\)D\(_3\) which are non-hypercalcaemic [8]. One such analogue is now widely used topically for the treatment of psoriasis [9]. However, no analogue has yet been found which has a specific effect on the parathyroid and no effect on serum calcium. For example, 22-oxacalcitriol was shown to be less hypercalcaemic in vivo in rats and also less effective on the parathyroids than 1,25(OH)\(_2\)D\(_3\) [8]. This is to be expected until a metabolite is discovered which has a different mechanism of action from that of 1,25(OH)\(_2\)D\(_3\) which acts as a classical sterol hormone. One possibility is a compound which has less affinity for the nuclear accessory proteins which are part of the receptor complex which binds to the target gene of 1,25(OH)\(_2\)D\(_3\).

It has been found that it is most effective and less hypercalcaemic to give 1,25(OH)\(_2\)D\(_3\) as larger boluses three times a week than for it to be given daily [10]. It is as effective for the 1,25(OH)\(_2\)D\(_3\) to be given orally or intravenously as would be expected from the pharmacokinetics of this readily absorbable polar compound [11]. What is intriguing is how in physiological or experimental situations where there are elevated endogenous serum concentrations of 1,25(OH)\(_2\)D\(_3\) there can still be very increased concentrations of PTH. For example, rats fed a low calcium diet have increased 1,25(OH)\(_2\)D\(_3\) and no suppression of PTH gene transcription [12]. This puzzle remains to be unravelled.

Phosphate and the parathyroid

The question that has long been asked is whether phosphate has an effect on the parathyroid which is independent of calcium and 1,25(OH)\(_2\)D\(_3\). The work of Kilav et al. [13] now provides convincing proof that phosphate does have an independent effect. Kilav et al. studied the expression of the PTH gene and serum PTH in rats fed phosphate-deficient diets and showed that hypophosphataemia led to a marked decrease in PTH mRNA and iPTH. A diet low in phosphate was associated with increased serum calcium and 1,25(OH)\(_2\)D\(_3\). They therefore bred second generation vitamin D-deficient rats and fed them diets low in phosphate, calcium and vitamin D. After 1 day of this diet these rats had hypophosphataemia with no increase in serum calcium or 1,25(OH)\(_2\)D\(_3\) and still had a decrease in PTH mRNA. In addition they showed that the effect of low phosphate was post-transcriptional unlike the effect of 1,25(OH)\(_2\)D\(_3\) which has a transcriptional effect. They thereby concluded that the effect of phosphate on the parathyroid was independent of the effect of calcium and 1,25(OH)\(_2\)D\(_3\) [13]. They have still not shown whether it is a direct effect or mediated by some other factor. This question remains a challenge for the future. There are other studies which also show that phosphate has an independent effect on the parathyroid.

Phosphate retention has long been considered to be important to the pathogenesis of the secondary hyperparathyroidism of chronic renal failure, and the resultant disabling renal osteodystrophy. Lopez-Hilker et al. [14] have shown in dogs with experimental chronic renal failure that phosphate restriction corrected their secondary hyperparathyroidism independent of changes in serum calcium and 1,25(OH)\(_2\)D\(_3\). They did this by placing the uraemic dogs on diets deficient in both calcium and phosphate, which led to reduced serum phosphate and calcium, with no increase in the serum 1,25(OH)\(_2\)D\(_3\), and despite this there was a 70% decrease in PTH. This study on the effect of a low phosphate diet on serum PTH suggested that phosphate had an effect on the parathyroid cell by a mechanism independent of its effect on serum 1,25(OH)\(_2\)D\(_3\) and calcium.

Clinical studies have demonstrated that phosphate restriction in patients with chronic renal insufficiency is effective in preventing the increase in serum PTH [10,15,16]. The mechanism of this effect was not clear, although at least part of it was considered to be due to changes in serum 1,25(OH)\(_2\)D\(_3\) concentrations, but a number of studies have shown that the effect of phosphate was independent of that of calcium and 1,25(OH)\(_2\)D\(_3\). In vitro [17] and in vivo [18] phosphate directly regulated the production of 1,25(OH)\(_2\)D\(_3\). A low phosphate certainly has a direct effect on cells in culture. Cells from a number of organs such as kidney, heart and liver, if maintained in a culture medium with a low phosphate concentration, have an increased transport of phosphate [19]. This is mediated by activation of a sodium phosphate co-transporter protein [20]. Initially there is an increase in activity of the transporters present in the cell membrane and then there is an increase in the number of transporters [21]. This is the result of the low phosphate but it is not known how the message of a low phosphate is recognized by the cell. Whatever the mechanism in vivo in the rat the effect of hypophosphataemia on the parathyroid cell is most dramatic leading to a large decrease in PTH gene expression and serum PTH. An increased serum phosphate decreases serum 1,25(OH)\(_2\)D\(_3\) and serum calcium by formation of calcium phosphate in the serum which is then deposited in bone and soft tissues. Therefore, phosphate undoubtedly plays a central role in the pathogenesis of secondary hyperparathyroidism, both by its effect on serum 1,25(OH)\(_2\)D\(_3\) and calcium and independently. What is clear from these studies is that the maintenance of a normal serum phosphate is important to the prevention of secondary hyperparathyroidism. This is often a difficult task for patients suffering from chronic renal failure.

Calcium and the parathyroids

The parathyroid is exceptional in that a change in extracellular calcium is reflected by corresponding changes in intracellular calcium and that a low calcium leads to PTH secretion. The mechanisms of the ionized calcium effect on the parathyroid are now more clear

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in that the parathyroid calcium receptor has been cloned by Brown et al. [22]. A high extracellular calcium activates the calcium receptor which then mobilizes calcium from intracellular sources and in some way inhibits the secretion of PTH. However, the effects of calcium on the parathyroid cell are not restricted to an effect on PTH secretion. Calcium also regulates PTH gene expression. Hypocalcaemia leads to a marked increase in PTH mRNA in vivo [12,23]. Weanling rats fed a low calcium diet for 3 weeks have a 10-fold increase in PTH mRNA and this effect is mainly due to increased PTH mRNA per parathyroid cell, that is the contribution of parathyroid hyper trophy is greater than that of parathyroid hyperplasia at least in the initial stages of secondary hyperparathyroidism [12]. This in vivo effect is not at the level of PTH gene transcription, as Naveh-Many et al. showed by performing nuclear transcript run-ons for PTH mRNA in parathyroid nuclei from rats fed low calcium diets. Despite a large increase in PTH mRNA there was no change in PTH gene transcription. Therefore the effect of calcium on PTH gene expression is post-transcriptional. In addition, in vivo the effect of calcium on PTH mRNA is that of hypocalcaemia to increase PTH gene expression and hypercalcaemia has no effect [12,24]. A high serum calcium of 18 mg/dl for up to 10 days in rats due to transplanted Walker osteosarcoma cells led to no decrease in PTH mRNA [24]. A number of other manoeuvres to increase serum calcium such as a high calcium diet also led to control levels of PTH mRNA. Therefore the effect of calcium on the PTH gene is that of hypocalcaemia to increase PTH mRNA and it is post-transcriptional. This is particularly important because there are a number of reports of in vitro studies using bovine PT cells in primary culture where an effect of high calcium had been observed. However, this does not represent a physiological state because the parathyroid calcium receptor is no longer present on cells in vitro at 24 hours [25]. In vitro studies on PTH mRNA were all performed well after 24 hours so the results must represent some other function of primary cultures. A similar caveat must be applied to the interpretation of the redox factor protein, refl, which has been shown to bind to a DNA sequence in the PTH gene promoter under the influence of an increased calcium [26]. What is of interest is the preliminary evidence from Hawa et al. [27] that there is a translational regulation of PTH synthesis. This would infer a further level of control of PTH production. Earlier studies had indicated that in secondary hyperparathyroidism there was a change in the calcium-PTH set point, with higher concentrations of serum calcium being needed to decrease PTH secretion than in controls. However, careful studies using more sensitive PTH radioimmunoassays and controlled changes in serum calcium show that there is no change in the calcium-PTH set point in chronic renal failure [28].

Calcium also regulates PTH synthesis at another level, namely at the level of parathyroid cell proliferation [29]. In patients with secondary hyperparathyroidism it is an obvious finding that there is a large increase in parathyroid cell number. This occurs in uraemic patients, in patients with vitamin D deficiency due to steatorrhoea, and patients with X-linked hypophosphataemia treated with large doses of phosphate and 1,25(OH)2D3. The only feature common to all these conditions is hypocalcaemia. So it is very probable that hypocalcaemia leads to parathyroid cell replication.

So calcium regulates the parathyroid cell at a number of levels and with different time sequences. Hypocalcaemia leads to increased PTH secretion in seconds and minutes, gene expression in hours and parathyroid cell number in weeks and months. The physiological result is that PTH maintains a tightly controlled serum calcium. With the persistent stimulation of hypocalcaemia secondary hyperparathyroidism results eventually with tremendous overgrowth of the parathyroids and pathological effects on bone, ostitis fibrosa cystica. In this situation there is often a need for subtotal parathyroidectomy and examination of parathyroid tissue removed at surgery has shown that most of these hyperplastic glands represent monoclonal growths. Therefore a reactive secondary hyperparathyroidism may develop into a multiple adenomatous neoplasia reminiscent of the primary hyperparathyroidism of multiple endocrine neoplasia, but of course with a completely different molecular pathogenesis.

**Regulation of parathyroid cell proliferation [30]**

Secondary hyperparathyroidism is characterized by an increase in parathyroid cell number, and PTH synthesis and secretion. It is still unknown as to what stimuli regulate parathyroid cell proliferation and how they do this. We have studied rats with dietary-induced secondary hyper- and hypoparathyroidism, rats given 1,25(OH)2D3 and rats after 5/6 nephrectomy for the presence of parathyroid cell proliferation and apoptosis [30]. Parathyroid cell proliferation was measured by staining for proliferating cell nuclear antigen (PCNA) and apoptosis by in situ detection of nuclear DNA fragmentation and correlated with serum biochemistry and PTH mRNA. A low calcium diet led to increased PTH mRNA and a 10-fold increase in parathyroid cell proliferation. A low phosphate diet led to decreased PTH mRNA and the complete absence of parathyroid cell proliferation. A low phosphate diet led to decreased PTH mRNA and the complete absence of parathyroid cell proliferation. 1,25(OH)2D3 (25 pmol/day x 3) led to a decrease in PTH mRNA, and unlike the hypophosphataemic rats there was no decrease in cell proliferation. There were no cells undergoing apoptosis in any of the experimental conditions. The secondary hyperparathyroidism of 5/6 nephrectomized rats was characterized by an increase in PTH mRNA and parathyroid cell proliferation which were both markedly decreased by a low phosphate diet. The number of PCNA-positive cells was increased by a high phosphate diet. Therefore hypocalcaemia, hyperphosphataemia and uraemia lead to parathyroid cell proliferation, and hypophosphataemia completely abolishes this
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effect. Injected 1,25(OH)2D3 had no effect. These findings emphasize the importance of a normal phosphate and calcium in the prevention of parathyroid cell hyperplasia.

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References

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