Phosphate and the parathyroid

Justin Silver[1] and Tally Naveh-Many[1]

[1]Minerva Center for Calcium and Bone Metabolism, Nephrology Services, Hadassah Hebrew University Medical Center, Jerusalem, Israel

The phosphate (Pi) retention in patients with chronic kidney disease leads to secondary hyperparathyroidism (2HPT). 2HPT is the physiological response of the parathyroid not only to Pi retention but also to decreased synthesis of $1,25(OH)_2$ vitamin D, and the attendant hypocalcemia. 2HPT is characterized by increased PTH synthesis, secretion, and parathyroid cell proliferation. Extracellular fluid (ECF) Ca^{2+} is recognized by the parathyroid calcium receptor and a small decrease in the ECF Ca^{2+} results in relaxation of the calcium receptor and allows the unrestrained secretion and synthesis of PTH and in the longer term, parathyroid cell proliferation. Both $1,25(OH)_2$ vitamin D and fibroblast growth factor 23 inhibit PTH gene expression and secretion. Secondary hyperparathyroidism can initially be controlled by a single therapeutic intervention, such as a Pi-restricted diet, a calcimimetic, or an active vitamin D analog. In this review we discuss the mechanisms whereby Pi regulates the parathyroid. Pi has a direct effect on the parathyroid which requires intact parathyroid tissue architecture. The effect of Pi, as of Ca^{2+}, on PTH gene expression is post-transcriptional and involves the regulated interaction of parathyroid cytosolic proteins to a defined *cis* acting sequence in the PTH mRNA. Changes in serum Ca^{2+} or Pi regulate the activity of *trans* acting interacting proteins in the parathyroid, which alters their binding to a defined 26 nucleotide *cis* acting instability sequence in the PTH mRNA 3′-untranslated region. The *trans* factors are either stabilizing or destabilizing factors and their regulated binding to the PTH *cis* acting element determines the PTH mRNA half-life. The responses of the parathyroid to changes in serum Pi are now being revealed but the sensing mechanisms remain a mystery.

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Correspondence: *Justin Silver, Minerva Center for Calcium and Bone Metabolism, Nephrology Services, Hadassah Hospital, PO Box 12000, Jerusalem 911220, Israel. E-mail: silver@huji.ac.il*

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Pi HOMEOSTASIS

Extracellular fluid (ECF) phosphate (Pi) concentration is regulated by a combination of local and humoral factors. The major humoral factors are the phosphatonins, fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH).[1] Both FGF23 and PTH are phosphaturic hormones and act to independently inhibit the activity of the renal sodium phosphate cotransporter, Na/Pi-2a, with resultant phosphaturia.[2] The local factors act as sensing mechanisms in different tissues, which determine the activity of the Na^+/Pi cotransporters.[3] The local tissue response to ECF Pi has been best characterized in the kidney, although it occurs also in both the duodenum and the parathyroid.[4–6] The kidney has the innate ability to detect changes in serum Pi and regulate the active reabsorption of Pi, an effect that is maintained in proximal tubule cell lines, and is independent of any hormonal affect.[7] The change in intracellular Pi then activates signal transduction and has local tissue effects, which may be physiological or pathological, depending on the concentration and duration of the Pi stimulus. Recently, it has been shown that the intestine detects the presence of increased dietary phosphate and rapidly increases renal phosphate excretion.[4] An increase in ECF Pi leads to an increase in FGF23 and PTH secretion. These effects are independent of any changes in $[Ca^{2+}]_o$ or $1,25(OH)_2$ vitamin D, which themselves regulate both PTH and FGF23 secretion, although in opposite directions.[8–10] A high $[Ca^{2+}]_o$ or $1,25(OH)_2$ vitamin D suppresses PTH secretion and stimulates FGF23 secretion.[9,11] The response to changes in Pi concentration implies a sensitive Pi-sensing system, the nature of which is a mystery.[12] Pi homeostatic mechanisms are well developed not only in mammals but also in unicellular organisms, both prokaryotes and eukaryotes. Growth in a nutrient medium without Pi results in the induction of genes that code for proteins responsible for Pi transport and secreted enzymes that would increase the supply of Pi in the medium and for those regulating the cell cycle.[13] The power of yeast genetics has provided an unparalleled strength to dissect out the regulatory pathways used by yeast in response to Pi depletion.[14] However, even in yeast, the Pi sensor remains to be identified.

Pi AND FGF23 EXPRESSION

Fibroblast growth factor 23 is predominantly secreted by osteocytes and is a major factor in the regulation of Pi homeostasis.[10,15] Dietary Pi loading leads to an increase in

bone FGF23 mRNA and serum FGF23. This effect is not a rapid response like that of the parathyroid calcium receptor (CaR) in response to changes in Ca^{2+} concentration or a hormone ligand and its receptor, but is rather only evident after longer time periods. It is not known how bone cells sense the increase in serum Pi. $1,25(OH)_2$ vitamin D also increases FGF23 synthesis and secretion by bone cells and it was shown that the vitamin D receptor expression in chondrocytes was necessary for this regulation.[10,16] Both *in vivo*, using mice with chondrocyte-specific inactivation of vitamin D receptor, and *in vitro* analysis showed that normal FGF23 production by osteoblasts or osteocytes is dependent on vitamin D receptor genomic action in chondrocytes.[16] Therefore, the vitamin D receptor signaling in chondrocytes regulates FGF23 synthesis.

An increase in serum calcium is another factor that increases FGF23 secretion.[9] In turn, FGF23 acts on the kidney to cause phosphaturia and a decreased synthesis of $1,25(OH)_2$ vitamin D and potentially corrects the high levels of Pi and $1,25(OH)_2$ vitamin D. FGF23 also regulates PTH gene expression and secretion. It acts on its receptor, Klotho-FGF receptor1c in the parathyroid, to cause a decrease in PTH mRNA levels and PTH secretion, an effect mediated by the mitogen-activated protein kinase pathway.[17] However, in chronic kidney disease (CKD), there are increased levels of serum FGF23 and PTH, indicating a resistance to the effect of FGF23 in the parathyroid in CKD.[18–21] The mechanism of the resistance of parathyroid to FGF23 remains to be explained.

Pi AND THE PARATHYROID

The parathyroid is geared to respond to a low serum Ca^{2+} by secreting PTH, which then acts on its target tissues to correct the serum Ca^{2+}.[22] The parathyroid senses serum Ca^{2+} through a membrane receptor, the G-protein-coupled receptor (GPCR), the CaR.[23] A high ECF Ca^{2+} activates the CaR to initiate signal transduction that inhibits PTH synthesis and secretion and parathyroid cell proliferation.[24] When the serum Ca^{2+} is decreased, more PTH is secreted, which then acts on its cognate G-protein-coupled receptor, the PTH1R, at its target tissues, bone and the renal tubule, and corrects the serum Ca^{2+}. PTH also causes phosphaturia and hence decreases serum Pi. A major hormone regulating Pi homeostasis is FGF23, which acts on the kidney to cause Pi loss and inhibits $1,25(OH)_2$ vitamin D secretion. However, FGF23 and PTH also share a direct interaction, in which FGF23 acts on the parathyroid to decrease PTH gene expression and secretion.[17,25] The trio is maintained in tempo by the action of $1,25(OH)_2$ vitamin D, which fine tunes PTH and FGF23 by increasing FGF23 and decreasing PTH. Pi in turn regulates parathyroid gland activity and PTH secretion independently of secondary changes in ECF Ca^{2+}, $1,25(OH)_2$ vitamin D, or FGF23, thus completing a network of endocrinological feedback loops (Figure 1).[26] These endocrinological feedback loops have been studied in animals with a normal renal function.

Pi REGULATES THE PARATHYROID INDEPENDENTLY OF CALCIUM AND $1,25(OH)_2$ VITAMIN D

The demonstration of a direct effect of high Pi on the parathyroid *in vivo* has been difficult. One of the reasons is that the various maneuvers used to increase or decrease serum Pi invariably lead to a change in the ionized Ca^{2+} concentration. In moderate renal failure, Pi clearance decreases and serum Pi increases; this increase becomes an important problem in severe renal failure. Hyperphosphatemia has always been considered central to the pathogenesis of secondary hyperparathyroidism (2HPT), but it has been difficult to separate the effects of hyperphosphatemia from those of the attendant hypocalcemia and decrease in serum $1,25(OH)_2$ vitamin D levels. However, it was shown by careful studies in dogs with experimental CKD that dietary Pi restriction prevented 2HPT.[27,28] Pi restriction corrected the 2HPT of CKD independent of changes in serum calcium and $1,25(OH)_2$ vitamin D levels.[28] Dietary restriction of both calcium and Pi led to lower levels of serum Pi and Ca^{2+}, with no increase in the low levels of serum $1,25(OH)_2$ vitamin D. Despite this, there was a 70% decrease in PTH levels. This study suggested that, at least in CKD, Pi affected the parathyroid cell by a mechanism independent of its effect on serum $1,25(OH)_2$ vitamin D and Ca^{2+} levels.[28] Therefore, Pi plays a central role in the pathogenesis of 2HPT, both by its effect on serum $1,25(OH)_2$ vitamin D and Ca^{2+} levels and, possibly, independently. These results were later substantiated in clinical studies that demonstrated that Pi restriction in patients with CKD prevented the increase in serum PTH levels.[29–33] The mechanism of this effect was not clear, although at least part of it was considered to be due to changes in serum $1,25(OH)_2$ vitamin D concentrations. In a study of patients with early CKD, Levin *et al.*[34] showed that low levels of $1,25(OH)_2$ vitamin D occur earlier in the course of estimated glomerular filtration rate (GFR) decline than do elevations in serum PTH levels. The increased serum PTH preceded the changes in serum Ca^{2+} or Pi. The low $1,25(OH)_2$ vitamin D levels might then lead to a secondary increase in PTH[11,35] that would lead to increased phosphaturia. As long as there are adequate renal reserves the augmented phosphaturia would prevent hyperphosphatemia. The time sequence of serum FGF23 levels during the induction of CKD and the subsequent progression of CKD remains to be studied. Serum FGF23 increases in patients with CKD stage. FGF23 was elevated at CKD stage 4 and 5 compared with CKD 1–2 in parallel with hyperphosphatemia.[21] It was suggested that high levels of FGF23 predict the development of hyperparathyroidism in dialysis patients.[36] The low levels of serum $1,25(OH)_2$ vitamin D may reflect a response to a direct effect of Pi on the renal synthesis of $1,25(OH)_2$ vitamin D. Pi directly regulated the production of $1,25(OH)_2$ vitamin D by kidney cells in culture[37,38] and *in vivo*.[30,39]

The effects of serum Pi on PTH gene expression and serum PTH levels are also independent of any changes in serum Ca^{2+} or $1,25(OH)_2$ vitamin D in rats with a normal