Secondary hyperparathyroidism and phosphate sensing in parathyroid glands

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Abstract: Retention of inorganic phosphate (Pi) and associated hyperphosphatemia are important in the development of hyperparathyroidism secondary to renal failure. The beneficial effect of a low-Pi diet in the prevention of hyperparathyroidism can be attributed to the decrease in PTH secretion. This effect of Pi may be mediated by specific molecules in the parathyroid cell membrane. A complementary DNA encoding a Na^+-Pi co-transporter, termed rat PiT-1, has been isolated from rat parathyroid. The amount of PiT-1 mRNA in the parathyroid is controlled by vitamin D and dietary Pi, which are the most important regulators of PTH secretion. The parathyroid Pi transporter may mediate the effects of extracellular Pi and PTH secretion in secondary hyperparathyroidism. In this study, we focus on the function of Na/Pi co-transporters in the parathyroid glands as inorganic Pi sensor. J. Med. Invest. 47 : 118-122, 2000

Key words : *parathyroid, phosphate, transporter, secondary hyperparathyroidism, vitamin D*

INTRODUCTION

Secondary hyperparathyroidism is generally present in uremia patients, and alterations in phosphate (Pi) homeostasis are important in its development and progression [1-4]. In renal insufficiency, the failure to control serum phosphate increases parathyroid hormone (PTH) secretion because of increased skeletal muscle resistance to PTH and suppression of serum calcitriol. Both of these factors adversely affect calcium homeostasis and contribute to the development of parathyroid gland hyperplasia [1, 2]. The enhanced phosphate burden in renal failure, which results in a continual demand for greater PTH secretion and a low calcitriol level, in addition to its effect on intestinal calcium absorption, may also contribute to a failure to suppress parathyroid gland growth [2, 3]. In contrast, PTH regulates the serum Pi concentration through its effect on the kidney to decrease Pi clearance and increase serum Pi, which becomes an important problem in severe renal failure [1-3].

Clinical studies have demonstrated that dietary Pi restriction in patients with chronic renal insufficiency is effective in preventing the increase in serum PTH levels [1]. A number of careful clinical and experimental studies have suggested that the effect of Pi on serum PTH levels is independent of changes in the levels of both serum calcium and 1,25-dihydroxyvitamin D_3 (1,25 $(OH)_2D_3$) [1-4]. In addition, Almaden et al. [5] showed that high Pi levels directly stimulated both PTH secretion from whole rat parathyroid glands in culture and PTH mRNA levels in human parathyroid tissue in culture. Slatopolsky *et al*. [6] showed similar findings for PTH secretion from rat parathyroid glands maintained in primary culture. These findings indicate that Pi regulates the parathyroid function directly. This effect of Pi may be mediated by specific molecules in the parathyroid cell membrane or by metabolic signals associated with increased cellular Pi concentrations. One such mediator may be a Na^+-dependent Pi co-transporter in the plasma membrane of parathyroid cells.

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Mammalian Na/Pi co-transporters

At least three types of Na/Pi co-transporter have been isolated from mammalian cells [7, 8]. Type I and type II transporters have been isolated from kidney cortex; they mediate Na^+-dependent Pi co-transport in the apical membrane of proximal tubular cells. Type III transporters were isolated as receptors for gibbon ape leukemia virus (GLVR1 or PiT-1) in mice and humans and amphotropic murine retrovirus (Ram-1 or PiT-2) in rats, and were shown to serve normal cellular functions as Na^+-dependent Pi co-transporters in several tissues [8]. PiT-1 and PiT-2 are approximately 60% identical in amino acid sequence, and exhibit no significant overall sequence homology with the type I or type II transporters. We isolated and characterized a Na/Pi co-transporter cDNA from rat parathyroid glands.

Cloning of a parathyroid Na/Pi co-transporter

In a previous study, we isolated Na/Pi co-transporter cDNA from rat parathyroid glands [9]. The protein encoded by this cDNA clone, rat PiT-1, showed Na-dependent Pi co-transport activity when expressed in *Xenopus* oocytes and appears to be the homologue of human and mouse PiT-1 (Glvr-1), Na/Pi co-transporters from mice and humans that also function as virus receptors (Figure 1). The rat PiT-1 sequence shows weak homology to *pho-4*$^+$, a gene implicated in phosphate uptake *in Neurospora crassa* and also distantly related to partially characterized genes from *Escherihi coli*, *Streptomyces haslstedii* and *Methanobacterium thermoautotrophicum*. PiT-1 and *pho-4*$^+$ display similar hydropathy plots, contain similar internal repeats, and are homologous in their primary structures. This family of high affinity Pi transporters allows cells to grow under restrictive conditions requiring a high-affinity uptake of Pi. Thus, PiT-1 is a member of an ancient Pi transporter gene family.

Characterization of PiT-1 in the parathyroid glands

To characterize the Na^+-dependent Pi cotransport system in rat parathyroid glands, we measured the initial Pi flux rate over a wide range of Pi concentrations [9]. In the absence of Na^+ in the incubation medium, the transport of Pi was significantly reduced, amounting to < 55% of the total uptake. The kinetic values for the Na^+-dependent Pi transport system in rat parathyroid glands were similar to those for PiT-1, suggesting that PiT-1 is a functional Na^+-dependent Pi transporter in rat parathyroid glands. The significant difference in Km values (89 $\pm$13 and 140$\pm$20 μM, $P<0.05$) observed between parathyroid glands and *Xenopus* oocytes could be the result of uncontrolled variables, such as membrane potential or post-translational modifications [9].

Regulation of PiT-1 in the parathyroid glands

The amount of PiT-1 mRNA in the thyroparathyroid tissue was reduced in vitamin-D-deficient animals compared with that in normal animals. When expressed relative to the amount of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, the administration of $1,25(OH)_2D_3$ to vitamin-D-deficient rats in-

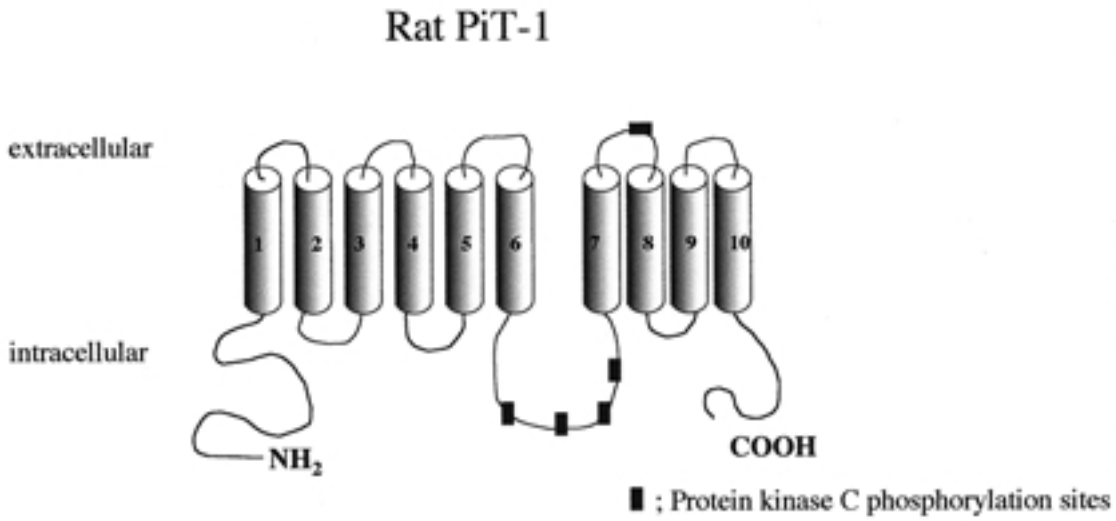

Fig. 1. The predicted structure of the parathyroid Na^+-Pi co-transporter PiT-1.
The 10 predicted transmembrane regions (M1-M10) are shown.

duced 2.3- and 3.3-fold increases in the amount of PiT-1 mRNA in these glands after 24 and 48 h, respectively [9].

Rats fed a low-Pi diet for 14 days showed a markedly lower plasma concentration of Pi than animals fed a high-Pi diet over the same period [9]. When expressed relative to the amount of GAPDH mRNA, the amount of PiT-1 mRNA in the thyroparathyroid tissue of rats fed the low- and control-Pi diets was 3.2 and 2.5 times that of the animals fed the high-Pi diet, respectively [9]. The level of PiT-1 mRNA in the parathyroid glands was also decreased in chronic renal failure (CRF) rats [9].

Sensing mechanisms of extracellular phosphate

i) Renal proximal tubular cells

Changes in the extracellular concentration of Pi modulate cellular function in a physiologically relevant manner. High extracellular Pi concentrations inhibit, while low concentrations stimulate, $1,25(OH)_2D_3$ synthesis in the renal proximal tubules. Extracellular Pi also modulates the renal tubular reabsorption of Pi, with reduced Pi enhancing and increased Pi diminishing the tubular reabsorption of Pi [7]. Extracellular Pi directly controls the apical Na/Pi co-transporter in the proximal tubular cells.

In opossum kidney proximal tubular cells (OK cells), a lowering of the medium Pi results in an increase in apical Na^+-Pi co-transport activity, and increasing the Pi concentration leads to lowered Pi uptake. It was found that the adaptive response to Pi deprivation can be elicited only when the apical side of the cells is in contact with a low Pi medium. The up-regulation of the apical Pi transport is apparent within 2h of replacement of the normal medium with a low Pi medium. Replacing the basolateral medium with a low-Pi medium has no effect on the apical adaptation. In addition, the basolateral Na^+-independent Pi transport does not undergo an adaptive response, whether the low Pi medium is on the apical side or the basal side. Based on these findings, it can be speculated that changes in the intratubular Pi concentration are the determining factors in the adaptive response [11]. This signal may stabilize the Na^+-Pi

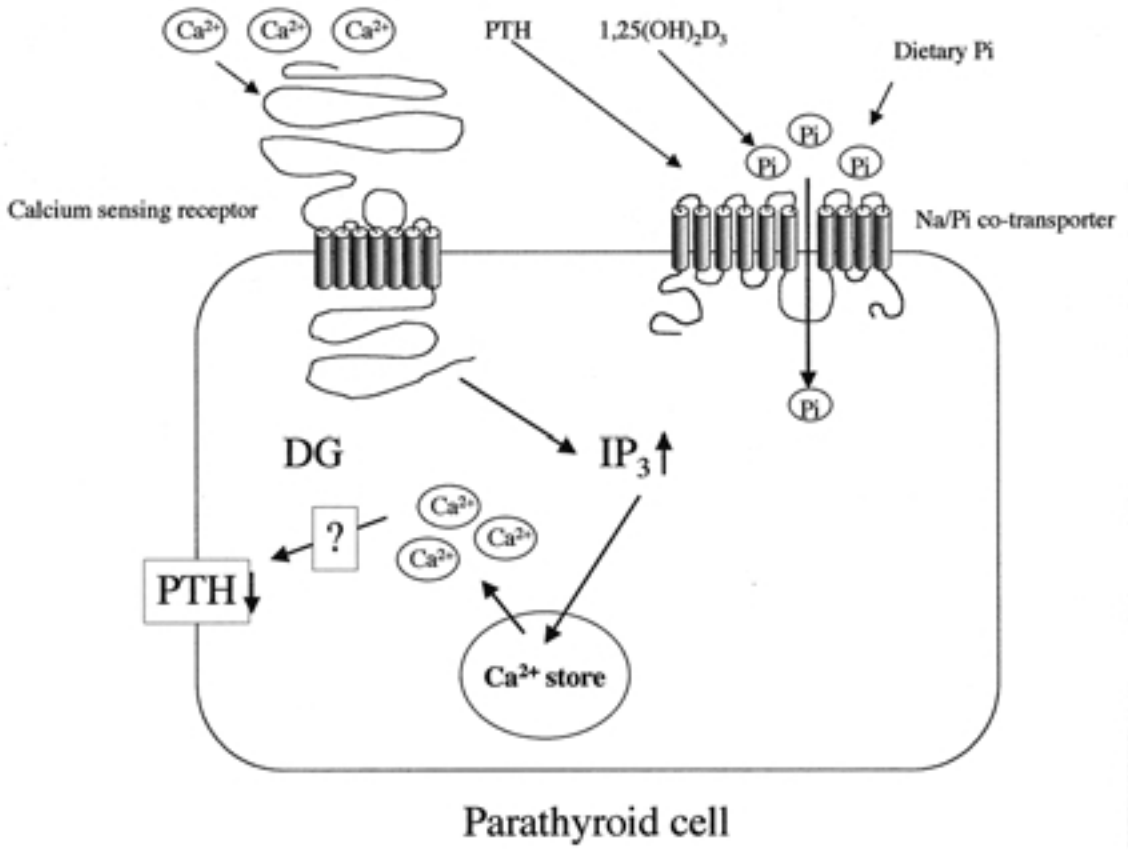

Fig. 2. Model of the possible coupling of cell surface Ca and Pi receptors to intracellular mediators and PTH secretion in parathyroid cells. High extracellular Ca and Pi bind to one or more types of cell surface receptors (transporters), each of which may interact with a separate intracellular effector system. High extracellular Pi may modulate the function of Na/Pi co-transporter, which controls the interaction between cellular inorganic phosphate and its binding protein [12].

co-transporter mRNA. These observations suggest that apical Na^+-Pi co-transporters may act as a Pi-sensing proteins in renal epithelial cells.

ii) Parathyroid cells

We suspect that a Na/Pi co-transporter may mediate the effects of extracellular Pi on the regulation of PTH synthesis. This transporter was regulated by $1,25(OH)_2D_3$ and by dietary Pi, but there is no evidence that the extracellular Pi needs to be up taken to exert its effect on the regulation of PTH synthesis. Further studies are needed to clarify whether PiT-1 functions as a cell-surface Pi-sensing protein in parathyroid cells.

Nuclear transcript run-on assays showed that the effect of low Pi was post-transcriptional, unlike the predominantly transcriptional effect of $1,25(OH)_2D_3$ on the PTH gene. Changes in the extracellular Pi concentration may modulate the stability of PTH mRNA (Figure 2). In addition, the effect of calcium on PTH secretion from dispersed bovine parathyroid cells occurs within seconds. However, the effect of Pi *in vitro* in cultured parathyroid glands requires about 4 h before any changes in PTH secretion are seen. Naveh-Many *et al*. [12] proposed that the effect on PTH gene expression is correlated with a decrease in PTH mRNA protein binding. This is in contrast to the effect of hypocalcemia, which increases this binding. These findings indicate that the final pathway of the effects of low Pi and low calcium on PTH mRNA share a common mechanism [12].

SUMMARY

The extracellular concentration of inorganic phosphate (Pi) is an important determinant of parathyroid cell function. The effects of Pi are mediated through specific molecules in the parathyroid cell membrane, one candidate molecule of which is a Na^+-dependent Pi co-transporter. A complementary DNA encoding a Na^+-Pi co-transporter, termed rat PiT-1, has been isolated from rat parathyroid glands. The amount of PiT-1 mRNA in the parathyroids of vitamin D-deficient rats was reduced compared with that in normal animals, and increased markedly after administration of 1,25-dihydroxyvitamin D3. In addition, the abundance of PiT-1 mRNA in the parathyroids was much greater in rats fed a low-Pi diet than in those fed a high-Pi diet. These studies indicate that rat PiT-1 contributes to the effects of Pi and vitamin D on parathyroid function as a Pi sensor.

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