Mechanisms of secondary hyperparathyroidism

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Silver, Justin, Rachel Kilav, and Tally Naveh-Many. Mechanisms of secondary hyperparathyroidism. Am J Physiol Renal Physiol 283: F367–F376, 2002; 10.1152/ajprenal.00061.2002.—Small decreases in serum Ca\(^{2+}\) and more prolonged increases in serum phosphate (Pi) stimulate the parathyroid (PT) to secrete parathyroid hormone (PTH), and 1,25(OH)\(_{2}\)D\(_3\) decreases PTH synthesis and secretion. A prolonged decrease in serum Ca\(^{2+}\) and 1,25(OH)\(_{2}\)D\(_3\) or increase in serum Pi, such as in patients with chronic renal failure, leads to the appropriate secondary increase in serum PTH. This secondary hyperparathyroidism involves increases in PTH gene expression, synthesis, and secretion, and if chronic, to proliferation of the PT cells. Low serum Ca\(^{2+}\) leads to an increase in PTH secretion, PTH mRNA stability, and PT cell proliferation. Pi also regulates the PT in a similar manner. The effect of Ca\(^{2+}\) on the PT is mediated by a membrane Ca\(^{2+}\) receptor. 1,25(OH)\(_{2}\)D\(_3\) decreases PTH gene transcription. Ca\(^{2+}\) and Pi regulate the PTH gene posttranscriptionally by regulating the binding of PT cytosolic proteins, trans factors, to a defined cis sequence in the PTH mRNA 3' untranslated region, thereby determining the stability of the transcript. PT trans factors and cis elements have been defined.

parathyroid hormone gene expression; posttranscriptional gene regulation; calcium; phosphate; vitamin D; chronic renal failure

THE PARATHYROID (PT) is an intriguing endocrine organ. It reacts in a unique way to a small decrease in concentration of a ligand, Ca\(^{2+}\), a divalent cation that is present in millimolar concentrations in the extracellular fluid (ECF), whereas in the cell it is present in 10,000-fold lower concentrations (67). This omnipresent ligand activates a seven-transmembrane, G protein-coupled receptor, the Ca\(^{2+}\)-sensing receptor (CaR) (15). The CaR's large extracellular portion and its method of recognizing ECF Ca\(^{2+}\) rendered its description as a Venus flytrap married to a serpentine membrane receptor (23). It uses acidic amino acids to bind the high concentrations of Ca\(^{2+}\) in the ECF rather than the EF-fingers used by intracellular Ca\(^{2+}\)-binding proteins that recognize and bind much lower Ca\(^{2+}\) concentrations. The trapped extracellular Ca\(^{2+}\) triggers a cascade of intracellular responses that not only prevent the secretion of PT hormone (PTH) but also degrade preformed hormone (34). When ECF Ca\(^{2+}\) is marginally decreased, the serpentine receptor is relaxed, and there is a quick release of its resident hormone, PTH. PTH then bursts forth to orchestrate the activation of its specific receptor on bone and kidney to release Ca\(^{2+}\) and complete the feedback loop. Not only does this peptide hormone prevent us from going into tetany, but it is also the potent treatment for that epidemic disease of aging populations, osteoporosis (57). However, when present in excess it destroys bone, particularly in patients with chronic renal failure and the frequent complication of secondary hyperparathyroidism (67). This review tries to provide perspective and depth to what is known and not known about the pathogenesis of secondary hyperparathyroidism, as well as highlight the large gaps in our knowledge.

Ca\(^{2+}\) AND THE CaR

The inverse relationship between serum Ca\(^{2+}\) and PTH secretion allows the sensitive maintenance of normal serum Ca\(^{2+}\). It is dependent on the sensing of serum Ca\(^{2+}\) by the CaR (14). High serum Ca\(^{2+}\) activates the CaR, whose message is transduced to the PTH secretory mechanism by phospholipase C (PLC)
and then indirectly to phospholipases D and A₂ (PLA₂) (40). Phospholipase D acts on phospholipids to release the biologically active compound phosphatidic acid. PLA₂ acts on membrane phospholipids to release arachidonic acid, which is then metabolized to active leukotriene metabolites that inhibit PTH secretion. Bourdeau et al. (9, 10) showed that high ECF Ca²⁺ increased the release of free arachidonic acid from PT cells and that the addition of free arachidonic acid or the products of its further metabolism suppressed PTH secretion. These results established a role for PLA₂ activity on regulated PTH release. Kifor et al. (41) have taken us more deeply into the PT cell by studying the effects of activation of the CaR on the mitogen-activated protein kinase (MAPK) pathway (41). The effect of the CaR is on the G₉/₁₁-phosphatidylinositol-PLC pathway to activate protein kinase C (PKC) as well as the G₁ pathway to decrease the activity of protein kinase A (PKA) and activate a tyrosine kinase. Secondary to these effects are the activation of PLA₂ and the subsequent release of free arachidonic acid and its metabolism to biologically active mediators such as hydroxyperoxyeicosatetranooic acid or hydroxyeicosatetranooic acid, which may then decrease PTH secretion (9, 10). They showed the centrality of MAPK to the effects of the PKC and G₁ pathways to phosphorylate and activate cytosolic (c)PLA₂ (41). To do this, they studied the regulation by the CaR on the phosphorylation of the MAPK, extracellular signal-regulated kinase (ERK)₁, and ERK₂, because they are known to phosphorylate cPLA₂. They utilized dispersed bovine PT cells and HEK-293 cells stably transduced with the CaR. Increased extracellular Ca²⁺ or a calcimimetic drug led to phosphorylation of ERK₁/2. The use of specific inhibitors showed that this effect was mediated by both the G₁ tyrosine kinase pathway as well as by the G₉/₁₁-phosphatidylinositol-PLC pathway to activate PKC. High Ca²⁺ increased serine phosphorylation of cPLA₂, which was inhibited by a selective MAPK inhibitor. Therefore, MAPK determines cPLA₂ activation (Fig. 1). In addition, these same researchers showed that the Ca²⁺ receptor’s COOH-terminal tail binds to filamin-A, and this may contribute to its localization in caveolae, link it to the actin-based cytoskeleton, and participate in the Ca²⁺ receptor-mediated activation of MAPK (36). Cohen et al. (22) showed by confocal Ca²⁺ imaging of PT cells that the interior of the PT cell is a nonhomogeneous medium and that an increase in the extracellular Ca²⁺ concentration produced changes in intracellular Ca²⁺ concentration, in both the same and opposite directions, in different parts of the PT cell. Therefore, there may be microdomains within the PT cell that determine the secretion of PTH after exposure to low ECF Ca²⁺ and not, as in other cells, after exposure to high ECF Ca²⁺.

The major physiological stimulus to PTH secretion is hypocalcemia. The implication is that physiological levels of serum Ca²⁺ partially activate the CaR and decrease PTH secretion. In the situation of hypocalcemia, the CaR is relaxed and PTH is secreted. The PT cell is therefore programmed to synthesize and secrete PTH continuously, and the CaR activates a brake on this process. Evidence in favor of such a mechanism is the paucity of PT secretory granules in the PT cell compared with other endocrine cells (33, 78). In addition, a major level of regulation of the PT is in the degradation of preformed PTH in the cell. Hypercalcemia results in >90% of PTH being degraded in the cell. This process results in COOH-terminal PTH fragments that are either released into the circulation or degraded in the PT, and the released amino acids are then incorporated into other proteins in the cell that are being translated (21). The mechanisms and regulation of PTH proteolysis in the PT remain to be clarified. Therefore, how does this contribute to our understanding of secondary hyperparathyroidism? In secondary hyperparathyroidism there is downregulation of the CaR protein, and for any increase in serum Ca²⁺ there is a less efficient inhibition of PTH secretion. As a result, for a particular serum Ca²⁺ concentration there is an enhanced secretion of PTH, which is the essence of the so-called “shift” in the Ca²⁺-PTH set
point of secondary hyperparathyroidism. This CaR downregulation occurs in the secondary hyperparathyroidism of chronic renal failure (12, 42), but it also occurs in other situations where there is PT cell proliferation, such as in PT primary adenomas as well as in transgenic mice with cyclin D1 targeted to the PT to cause hyperparathyroidism (38). Furthermore, in experimental chronic renal failure, calcimimetics, which activate the CaR, prevent the proliferation of PT cells and secondary hyperparathyroidism (75). Therefore, the expression and activity of the CaR are major determinants of the function of the PT cell, and its down-regulation is important to the development of secondary hyperparathyroidism. Downregulation of the expression of the 1,25(OH)2D3 receptor is also present in secondary hyperparathyroidism and may also contribute to the development of secondary hyperparathyroidism (29, 66). It certainly would be important to the failure of response to administered 1,25(OH)2D3.

**P, AND PTH SECRETION**

High serum Pi is a major stimulus to secondary hyperparathyroidism. In vitro this effect demands intact tissue architecture as it only occurs in tissue slices or whole glands in culture and not in isolated cells (5, 58, 71). The laboratory of Almaden et al. (3) have shown that the effect of high Pi to increase PTH secretion is due to a decrease in cPLA2 activity. Almaden et al. (2) studied the effect of Pi on intracellular Ca2+ and arachidonic acid production in the PT. In PT tissue incubated with either a Ca2+ ionophore, which increases Ca2+ influx across the cell membrane, or thapsigargin, which releases Ca2+ from intracellular stores, there was an increase in arachidonic acid production. This increase in arachidonic acid production was associated with an inhibition of PTH secretion, suggesting that cPLA2 is activated by the elevation in intracellular Ca2+ levels (2). Low serum Pi, both in vitro and in vivo has been shown to increase the intracellular Ca2+ concentration in a number of cell lines and tissues (28, 83). In the presence of either the Ca2+ ionophore or thapsigargin, high Pi was no longer able to decrease arachidonic acid levels, which were in fact increased (2). The ECF Pi concentration is reflected in a similar intracellular Pi level, and therefore the high serum Pi in chronic renal failure patients would result in a high intracellular Pi concentration in the PT. The increased intracellular Pi may then inhibit the release of intracellular Ca2+ from internal cellular stores such as the mitochondria or endoplasmic reticulum. Therefore, in patients with secondary hyperparathyroidism due to hyperphosphatemia, the increase in intracellular Ca2+ may then be the final messenger for the effect on cPLA2 and increased PTH secretion. However, much of the effect of both Ca2+ and Pi is due to an effect on PTH mRNA levels and its translation into PTH, which are discussed below.

**Mg2+ AND PT SECRETION**

Mg2+ also regulates PTH secretion. Mg2+ acts on the CaR with a lower affinity than Ca2+ (13). Clinically, it has long been recognized that patients with chronic hypomagnesemia are only able to increase their secretion of PTH after the serum Mg2+ has been corrected. The effect of low Mg2+ is due to its action on the intracellular side of the CaR, at the CaR-G protein interface. A decrease in Mg2+ concentration increased the rate of binding of the CaR’s GTPγS binding to recombinant Goi protein (61). In addition, Mg2+ inhibited the basal guanine nucleotide exchange of wild-type Goi GTP-binding protein but not of a Goi mutant with impaired Mg2+ binding. Therefore, the paradoxical block of PTH release under Mg2+ deficiency is mediated through a novel mechanism involving an increase in the activity of Go subunits of heterotrimeric G proteins.

**PTH GENE EXPRESSION**

The elucidation of how the PTH gene is regulated is intriguing and is of interest to understanding the pathogenesis of secondary hyperparathyroidism. This is because so much of the regulation of the PT is at the level of gene expression. The PT has a limited amount of preformed secretory granules containing mature PTH. The PTH in these granules is itself under regulatory control. In the face of persistent hypercalcemia, there is a rapid degradation of the mature PTH in the PT cell. With the stimulus of hypocalemia there is a rapid secretion of PTH that is rapidly renewed by the synthesis of new hormone. We have shown the mechanism of this regulation in vivo to be mainly posttranscriptional by an increase in PTH mRNA stability (43, 51). This is in contrast to the effect of 1,25(OH)2D3 to markedly decrease PTH gene expression, which is a transcriptional effect (69). Hypercalcemia does not decrease PTH mRNA levels any lower than normocalcemia (55). This is in contrast to the effect of hypercalcemia to decrease PTH secretion.

It all started with 1,25(OH)2D3 and the PT. In 1985, we showed in vitro that 1,25(OH)2D3 decreases PTH gene expression, and in the following year our laboratory showed in vivo in rats that 1,25(OH)2D3 dramatically decreased PTH gene transcription (69, 70). Studies by Slatopolsky et al. (72) in humans and many other studies have demonstrated the mechanism and efficiency of the effect. Of course, 1,25(OH)2D3 and its analogs are the mainstays for the prevention and treatment of secondary hyperparathyroidism in patients with renal failure. Of interest, 1,25(OH)2D3 receptor knockout mice have secondary hyperparathyroidism, which can be corrected by a diet rich in Ca2+ (47). This implies that the effect of vitamin D deficiency to cause secondary hyperparathyroidism is at least largely due to the secondary Ca2+ deficiency rather than due to the lack of effect of vitamin D on the PT itself. However, the effect of 1,25(OH)2D3 and its analogs on the PT are so potent that it is difficult to imagine that there is no physiological role for vitamin D on the PT.
What about Pi and Ca2+? Raised serum Pi and decreased serum Ca2+ are well-documented factors in the pathogenesis of secondary hyperparathyroidism. Interest in the biological effect of Pi is heightened by the large increase in mortality, due to cardiovascular complications, in patients with high serum phosphates (8). In our laboratory, we asked how Pi and Ca2+ regulate PTH gene expression. Studies by Kilav et al. (43) have shown that the effect of low Pi on PTH gene expression is independent of the attendant changes in serum Ca2+ and 1, 25(OH)2D3, with similar conclusions for the effect of high Pi from Hernandez et al. (35). In addition, in vitro Pi has a direct effect on PTH secretion, as long as the tissue architecture remains intact (4, 58, 71).

We utilized in vivo models of diet-induced Ca2+ and Pi deficiency to study how Ca2+ and Pi regulate the PT. The first finding was that despite the dramatic differences in PTH mRNA levels and serum PTH, there was no difference in their nuclear transcription rates (43, 51). Therefore, Ca2+ and Pi regulate PTH gene expression posttranscriptionally. Posttranscriptional gene regulation usually involves the binding of cytosolic proteins to the 3'-untranslated region (UTR) of mRNAs. By ultraviolet cross-linking and RNA electrophoretic mobility shift assay (REMSA), we showed that the effect of low Pi on PTH gene expression is due to the binding of proteins to the 3'UTR. We showed that the 3'UTR sequences seen by ultraviolet cross-linking are involved in determining RNA stability. In a particularly instructive experiment, we depleted all the 3'UTR binding proteins from the PT cytosolic extract. To do this, we added excess PTH RNA 3'-UTR, or a smaller transcript of 63 nt that is sufficient for binding, to the degradation assay of PTH RNA with PT proteins. This resulted in a rapid degradation of the PTH RNA, suggesting competition for the stabilizing proteins (51). Therefore, AUF1 is a protein that binds to the PTH mRNA 3'-UTR and stabilizes the PTH transcript. A model depicting the posttranscriptional regulation of the PTH transcript by Ca2+ and Pi is shown in Fig. 2.

Studies of protooncogene mRNAs, such as c-myc and c-fos, have established a role for AUF1 in AU-rich element (ARE)-directed mRNA decay that is based on its affinity for different AREs (32). The role of AUF1 in mRNA decay is not restricted to protooncogenes. The developmental immaturity of neonatal phagocytic function is associated with a shorter half-life of granulocyte-macrophage colony-stimulating factor (GM-CSF) mRNA. In vitro, the decay of the GM-CSF in mononuclear cells was accelerated by protein fractions enriched for AUF1 (17). Moreover, this accelerated ARE-dependent decay of the GM-CSF 3'-UTR was attenuated by immunodepletion of AUF1, thereby demonstrating that the in vitro RNA decay is ARE and AUF1 dependent (17). The ARE destabilizing function in K562 cells was dramatically impeded during heme-induced erythroid differentiation (48). Ectopic expression of heterogeneous nuclear ribonucleoprotein (RNP) D/AUF1 in hemin-treated K562 cells restored the rapid decay directed by the ARE. Therefore, AUF1 has a specific cytoplasmic function as an RNA destabilizing protein in the ARE-mediated decay pathway.

In contrast to the role of AUF1 in the rapid degradation of mRNAs, it may have a role in the stabilization of other mRNAs, such as α-globin mRNA. AUF1 was identified as one of the proteins, which together with two other proteins, αCP1 and αCP2, binds to the 3'-UTR of the α-globin mRNA. Together, they regulate the erythocyte-specific accumulation of α-globin mRNA. Alone, none of these proteins can bind the α-globin 3'-UTR, and they only bind when they are complexed with the other proteins of the α-complex (45). It is now clear that AUF1 binds PTH mRNA 3'-UTR and determines PTH mRNA stability. The PTH mRNA 3'-UTR has a region that is rich in adenosine and uridine but does not have the classic ARE configuration. The PTH
mRNA ARE is an example of a regulatory element that is stabilized by AUF1 and other PT cytosolic RNA-binding proteins. RNA-protein binding regulates PTH mRNA levels in response to changes in serum Ca\(^{2+}\) and Pi. The role of AUF1 in the regulation of PTH mRNA stability in response to changes in serum Ca\(^{2+}\) and Pi remains to be determined.

We then concentrated on defining the cis sequence in the PTH mRNA 3'-UTR to which the trans acting PT proteins bind and that determines the stability of the PTH transcript (44). We have identified the minimal sequence for protein binding in the PTH mRNA 3'-UTR and determined its functionality. A minimum sequence of 26 nt was sufficient for RNA-protein binding and competed for binding of the full-length 3'-UTR by REMSA. Antisense oligonucleotides to different regions of the conserved RNA element further identified this binding. The element’s sequence was preserved among species. The rat PTH mRNA 3'-UTR is 234 nt long. Sequence analysis of the PTH mRNA 3'-UTR of different species revealed a preservation of the 26-nt core protein-binding element in rat, murine, human, and canine 3'-UTRs. In particular, there is a stretch of 14 nt within the element that is present in all four species. In the 26-nt element, the identity among the species varies between 73 and 89%. The conserved sequence suggests that the binding element represents a functional unit that has been evolutionarily conserved, but more detailed analysis in many species is required before such a conclusion can be accepted. To study the functionality of the sequence in the context of another RNA, a 63-bp cDNA PTH sequence consisting of the 26-nt core and flanking regions was fused to the growth hormone (GH) cDNA. There is no PT cell line, and therefore an in vitro degradation assay was used to determine the effect of PT cytosolic proteins on the stability of RNA transcripts for PTH, GH, and a chimeric 63-nt GH-PTH. The PTH transcript was stabilized by PT proteins from rats fed a low-Ca\(^{2+}\) diet and destabilized by low-P\(_i\) PT proteins, similar to the PTH full-length transcript. Therefore, the 63-nt protein-binding region of the PTH mRNA 3'-UTR is both necessary and sufficient to regulate RNA stability and to confer responsiveness to changes in PT proteins by Ca\(^{2+}\) and P\(_i\) (44).

**Fig. 2. Model of the parathyroid hormone (PTH) mRNA 3'-untranslated region (UTR) and the PTH cytosolic proteins that interact with the 3'-UTR. The PTH proteins contain both protective factors (blue), measured by ultraviolet cross-linking, and degrading factors (ribonucleases; red), measured by an in vitro degradation assay. In normal rats, the basal levels of PTH mRNA are determined by a balance between the protective and degrading factors in the cytoplasm. In hypocalcemia, there is an increase in PTH mRNA associated with an increase in the binding of protective factors, which leads to a more stable transcript. In hypophosphatemia, there is a decrease in protective factors, which leads to a less stable transcript and a decrease in PTH mRNA levels. An, poly A tail.**

**BINDING OF PTH mRNA TO MICROTUBULES**

The 3'-UTR of mRNAs binds proteins, which determine mRNA stability, translation, and localization. We had shown that the 3'-UTR of PTH mRNA specifically bound cytoplasmic proteins and isolated one of these proteins by affinity chromatography as AUF1. We also screened an expression library for proteins that bound the PTH mRNA 3'-UTR, and the sequence of one clone was identical to dynein light chain [relative mass (M\(_r\)) 8,000] (LC8) (27). LC8 is part of the cytoplasmic dynein complexes that function as molecular motors which translocate along microtubules (46). Recombinant LC8 bound PTH mRNA 3'-UTR by REMSA. We showed that PTH mRNA colocalizes with polymerized microtubules in the PT gland, as well as with a purified microtubule preparation from calf brain, and this was mediated by LC8. This was the first report of a dynein complex protein binding an mRNA. In situ hybridization of rat PT tissue showed that PTH mRNA is localized to the periphery of the cell. Administration of paclitaxel in vivo to rats, which disrupts the microtubule structure, led to a marked decrease in the peripheral localization of PTH mRNA (unpublished observations). We suggest that the peripheral localization of PTH mRNA is due to its binding to LC8 and microtubules. Dynein light chain is also involved in targeting swallow and bicoid RNA to the anterior pole of *Drosophila* oocytes (65). Therefore, the dynein complex may be the motor for the transport and localization of mRNAs in the cytoplasm and the subsequent asymmetric distribution of translated proteins in the cell.
In eukaryotic cells, most cytoplasmic transport processes depend on cytoskeletal filaments. This is well established for the active transport of chromosomes, membranous organelles, and some large protein complexes. Force-producing ATPases (motor proteins) attach to the object to be moved and then “walk” along a filament, overcoming the resistance to movement imposed on large objects by the gel-like nature of cytoplasm (64). The relatively small size of an mRNA suggests that random diffusion and specific anchoring to the cytoskeleton in target areas might suffice for localization. In cells, however, mRNAs can complex with many proteins to form large RNP particles. Perhaps because of RNP size and/or requirements for efficiency, the localization of some mRNAs requires motor proteins, suggesting that the cytoskeletal filaments are actually used as tracks for active transport (16, 19). The PTH transcript may also utilize this mechanism to allow the more efficient utilization of its template at the periphery of the cell, for translation into PTH, which would then be available for rapid secretion. This hypothesis needs to be rigorously tested.

PT CELL PROLIFERATION

PT cells divide infrequently (59). However, the PT cell retains the latent ability to proliferate into large hyperfunctioning glands in a number of clinical conditions. A common situation is that of the secondary hyperparathyroidism in most patients with chronic renal failure. Primary hyperparathyroidism affecting all PT glands may be due to inactivating mutations in the menin gene such as the menin gene in MEN1 (1, 20), the gene encoding for the retinoblastoma protein (25), or activating mutations of the RET protooncoprotein (MEN2a) (30, 53). Mutations in the menin gene are found in 20% of single PT adenomas (6, 25). Other PT adenomas have been found to have a chromosomal translocation, whereby the PT promoter drives a translocated sequence, which was found to code for cyclin D1 (52).

The PT is geared to respond to hypocalcemia with an increase in PTH secretion in seconds and minutes, an increase in PTH mRNA levels in hours, and an increase in PT cell proliferation in the longer term. With hypocalcemia the CaR is relaxed and PTH secretion is not restrained. Therefore, without the CaR there would be a constitutive secretion of PTH, a finding that occurs in mice with knockouts of the CaR and patients with mutations in the CaR, FHH (37, 60). Uremic rats given calcimimetic agents that bind to the CaR had a decreased PT cell proliferation, demonstrating the role of the CaR in PT cell proliferation (75). First, what factors have the potential to stimulate the PT cell to leave its dormant state in G0 and enter the cell cycle and by what mechanism does this take place? These have been best characterized for patients with secondary hyperparathyroidism due to chronic renal failure and X-linked hypophosphatemia treated with excess P1. What emerges from these studies is that persistently low serum Ca2+ or high serum P1 levels are the major factors leading to PT cell proliferation (54). 1,25(OH)2D3 therapy directly decreases PTH gene transcription and PT cell proliferation (24, 56). However, vitamin D deficiency alone probably causes PT cells to proliferate because of the secondary chronic hypocalcemia (47).

Ca2+ is the major regulator of the PT at the levels of secretion, gene expression, and cell proliferation. In vivo, hypocalcemia leads to a profound increase in PT cell proliferation (54), and in vitro studies have been performed to investigate the mechanism. However, it is difficult to extrapolate from in vitro studies in PT cells. In primary cultures of bovine PT cells, there is down-regulation of the CaR (12, 50). After 24 h in culture, CaR mRNA and protein are present only in very low concentrations on the PT cells in primary culture, and at later time intervals, not present at all. There have been studies in a cell line derived from rat PT showing that Ca2+ regulates PT cell proliferation (11) and there are changes in cyclin D1 mRNA, not cyclin D2 and D3, after changes in medium Ca2+ concentration (7). Sakaguchi et al. (62, 63) showed that these cells expressed acidic fibroblast growth factor (aFGF) and expression of both aFGF mRNA and peptide was suppressed by calcium. Thymidine incorporation was stimulated by decreasing extracellular Ca2+ concentrations, and cell growth was also stimulated by low Ca2+ (62, 63). However, these cells do not secrete PTH. They do secrete PTH-related peptide, but this is not Ca2+ dependent. Thus their relevance to the PT is probably marginal.

Rats fed a low-Ca2+ diet become hypocalcemic, secrete more PTH, and have increased levels of PTH mRNA (56). In addition, their PT cells are hypertrophic, as studied by stereoscopic electron microscopy (73, 78), and there is an increase in the number of cells that are proliferating (54). The number of proliferating cell nuclear antigen (PCNA)-positive cells, as a measure of cell proliferation, in weanling rats fed a low-Ca2+ diet for 10 days increased sixfold. After 21 days on a low-Ca2+ diet, the rats showed a 3.6-fold increase in PCNA-positive cells, which correlated with a 5-fold increase in PTH mRNA levels (54). A high-P1 diet led to a moderate increase in PCNA-positive cells, with a similar increase in PTH mRNA levels. What was particularly striking was the effect of a low-P1 diet (54). After 21 days of a low-P1 diet, the rats had no PCNA-positive cells, which correlated with a 75% decrease in PTH mRNA levels.

CHRONIC RENAL FAILURE

Naveh-Many et al. (54) studied rats with experimental uremia and showed that there was an increase in PT cell proliferation compared with control rats. A high-P1 diet increased, and a low-P1 diet dramatically decreased, the number of proliferating PT cells. These findings emphasize the importance of normal serum P1 and Ca2+ in the prevention of PT cell hyperplasia. Similar results were found by Yi et al. (81). They showed that rats with experimental uremia had an increase in serum PTH, PTH mRNA, and PT cell proliferation, all of which were prevented by mild dietary...
phosphorus restriction. 1,25(OH)₂D₃ may have a role in regulating PT cell proliferation in chronic renal failure in addition to its role in decreasing PTH gene transcription. Szabo et al. (74) showed that the thymidine incorporation into isolated PT glands from uremic rats was decreased by prior treatment with 1,25(OH)₂D₃. Dusso et al. (26) studied P₁-restricted 5/6 nephrectomized rats (26). They showed that PT-p21 mRNA and protein increased by day 2, independently of changes in serum 1,25(OH)₂D₃, and remained higher than in the rats’ high-P₁ counterparts for up to 7 days. The PT hyperplasia of the high-P₁ group could not be attributed to a reduction in PT-p21 expression from normal control values. Instead, PT-transforming growth factor (TGF-α) protein was higher in uremic rats compared with normal controls and increased further with high dietary P₁ intake. PT levels of PCNA correlated inversely with p21 and directly with TGF-α. It has also been shown in human PTs that proliferation correlates with an increase in TGF-α levels (31). These findings suggested that low-P₁ induction of p21 could prevent PT hyperplasia in early uremia, whereas high-P₁ enhancement of TGF-α may function as an autocrine signal to stimulate growth further (26). Cozzolino et al. (24) showed that the PT cell hyperplasia in rats with experimental chronic renal failure was decreased by a high dietary Ca²⁺ or treatment with 1,25(OH)₂D₃. There was an increase in PT p21 expression, and the high-P₁-induced increase in TGF-α content was prevented (24), similar to the effects of P₁ restriction.

The PT cell proliferation in hypocalcemic rats can be prevented by compounds that inhibit the activation of the endothelin receptor (39). It is difficult to define the sequence of events and factors that lead to PT cell proliferation.

Fig. 3. Regulation of PTH proliferation, gene expression, and secretion. Cyclin D1, driven by the PTH promoter, and inactivating mutations of the menin gene are known to cause PT adenomas; germ-line mutations of the latter cause MEN1. The very rare PT carcinomas show lack of expression of the retinoblastoma protein (pRb). Activating mutations of the RET protooncogene result in MEN2a. Low serum Ca²⁺ leads to a decreased activation of the CaR and results in increased PTH secretion (blue dots), PTH mRNA stability, and PT cell proliferation. High serum P₁ leads to similar changes in all these parameters. Endothelin and transforming growth factor (TGF-α) are increased in the PTs of proliferating PT glands. 1,25(OH)₂D₃ decreases PTH gene transcription markedly and decreases PT cell proliferation. PTH mRNA stability is regulated by PT cytosolic proteins (trans factors; blue) binding to a short defined cis sequence (pink) in the PTH mRNA 3'-UTR and preventing degradation by ribonucleases (red). One of these protective proteins is the adenosine-uridine binding protein (AUF1). In hypocalcemia there is more binding of the trans factors to the cis sequence, leading to a more stable transcript. Low serum P₁ leads to much less binding and a rapidly degraded PTH transcript. Figure is reproduced from Ref. 68 with permission from the American Society of Clinical Investigation.
proliferation because in the experimental models available only a small percentage of the cells enter the cell cycle. Imanishi et al. (38) created transgenic mice with the cyclin D1 gene specifically expressed in the PT, relying on a 5.1-kb upstream region of the PTH gene to specifically target the transgene to the PT cell. As expected, the transgenic mice developed hyperparathyroidism with large hyperplastic and, in some cases, adenomatous glands. These mice were then used to study in vivo parameters of PTH physiology. PTH secretion, as measured by the concentration of serum Ca²⁺ needed to half-maximally suppress PTH secretion (Ca²⁺ set point), was increased in the mice with hyperparathyroidism, similar to the findings in patients with primary or secondary hyperparathyroidism. They also demonstrated a decrease in the expression of the CaR protein in the hyperplastic PTs, as has been found in patients with hyperplastic PTs. The CaR has been shown in Rat-1 fibroblasts to stimulate ERK1 kinase activity and cellular proliferation (49). This mechanism also explains high-Ca²⁺-induced growth in osteoblasts (80). In the PT, stimulation of the CaR by a high ECF Ca²⁺ concentration leads to a decrease in cellular proliferation. The uniqueness of the PT’s response remains to be explained.

A further mechanism by which PT cell number might be regulated is by inducing apoptosis. This has been studied in the PTs of hypocalcemic rats as well as in rats with experimental uremia fed different diets (54). Apoptosis was determined by the deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling method, which detects nuclear DNA fragmentation in situ. In no situation were apoptotic cells detected in the PTs. Similar negative findings were found in mature rats (76). However, in human PT adenomas, apoptotic cells were demonstrated, and this apoptosis correlated with the number of cells proliferating, as measured by Ki-67 immunoreactivity (77). Moreover, in a study of the PTs of uremic patients with secondary hyperparathyroidism, convincing evidence of apoptosis was documented (82). However, the number of apoptotic cells in the PTs of uremic rats is very small and increases in association with enhanced mitotic activity (18). Therefore, PT cells have the latent ability not only to proliferate but also to apoptose, but the mechanisms responsible for PT apoptosis are not known.

CONCLUSION

In diseases such as chronic renal failure, secondary hyperparathyroidism involves abnormalities in PTH secretion and synthesis and PT cell proliferation. Progress has been made in understanding how Ca²⁺, P₃, and vitamin D regulate the synthesis and secretion of PTH as well as the proliferation of the PT cells (Fig. 3). A more complete understanding of how the PT is regulated at each level will help in the devising of a rational therapy for the management of such conditions.

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