The Role of 1,25-$(OH)_2D_3$ in Regulating Parathyroid Gland Function (42914)

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Parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) (the biologically active metabolite of vitamin D_3) are the two major hormones responsible for the maintenance of calcium homeostasis. In carrying out their biologic actions, the two hormones interact with the kidney, bone, and gastrointestinal tract (1). Parathyroid hormone enhances calcium reabsorption in the distal renal tubule and decreases phosphate absorption by the proximal tubule, and 1,25-$(OH)_2D_3$ enhances calcium absorption by the intestine by a mechanism that is still not completely understood. In concert, a peptide hormone (PTH) acts with a 9,10-secosterol (1,25-$(OH)_2D_3$) to mobilize calcium from bone into the extracellular fluid. PTH also enhances the production of 1,25-$(OH)_2D_3$ by the kidney through activation of the 1 α-hydroxylase enzyme which is responsible for converting 25-$(OH)D_3$ to 1,25-$(OH)_2D_3$, the final activating step for vitamin D_3. What has been less clear are the effects of 1,25-$(OH)_2D_3$ on parathyroid gland function and specifically on PTH synthesis and secretion. Recent studies (2–4) have provided considerable clarification of this issue and emphasized a key role for 1,25-$(OH)_2D_3$ in regulating parathyroid gland function.

Nuclear receptors for 1,25-$(OH)_2D_3$, similar to receptors for steroid hormones, have been identified in target tissues for the hormone, namely, the intestine and bone (5, 6). Similar high affinity receptors have been demonstrated in parathyroid cells from the chicken and other species (7–9). Following the administration of [^{3}H]1,25-$(OH)_2D_3$ to rats and chicks, there is marked accumulation of the active metabolite in the nuclei of parathyroid cells. The 1,25-$(OH)_2D_3$ receptor has now been cloned and sequenced; it contains DNA- and ligand-binding domains analogous to steroid receptors that affect gene expression (10).

There has previously been confusion about the potential role of 1,25-$(OH)_2D_3$ in parathyroid gland function, with reports in the literature suggesting that the active metabolite suppresses, stimulates, or has no effect on PTH release (11–15). Studies from our laboratory provide direct evidence for a role of 1,25-$(OH)_2D_3$ in regulating PTH gene expression and ultimately the secretion of PTH (2–4).

Materials and Methods

Tissue culture medium (BME), trypsin-EDTA, fetal calf serum, and penicillin-streptomycin were obtained from Grand Island Biological Co., Grand Island, New York. Multidish culture plates were obtained from Nunc, Denmark and insulin, transferrin, and selenium from Collaborative Research, Lexington, Massachusetts. Radioactive isotope, ^{125}Na iodide, and ^{32}P were obtained from Amersham, Arlington Heights, Illinois and guanidine thiocyanate from Fluka Chemicals, Hauppauge, New York. PTH peptides were from Beckman, Palo Alto, California and Bachem, Torrance, California; 1,25-$(OH)_2D_3$ was a generous gift from Dr. M. Uskokovic, Hoffman-LaRoche Laboratories, Nutley, New Jersey.

Primary Tissue Culture. After digestion of bovine parathyroid tissue with collagenase as described (3), parathyroid cell number and viability were determined and cells initially plated at a density of 1–2 $\times$ 10^6 cells/ml. The culture medium was Eagle's basal medium with 10% fetal calf serum, 1% penicillin-streptomycin, 1.0 m*M* magnesium, 1.25 m*M* calcium, and insulin (5 μg/ml), transferrin (5 μg/ml), and selenium (5 ng/ml). The medium was buffered with 20 m*M* Hepes buffer and 2.2 mg/ml of $NaHCO_3$ to pH 7.5. The cells were established in culture for 48 to 72 hr prior to initiation of studies in which vitamin D metabolites were used, and each experimental plate was cultured in triplicate.

Extraction of Cytoplasmic RNA and Measurement of mRNA. Cells were removed from culture plates at different time intervals, with cellular RNA being extracted with phenol/chloroform. Northern blot hybridization (16) and dot blot hybridization (17) were used for quantitation of mRNA as a percentage of total RNA (estimated by absorbancy at 260 nm). RNA for dot blots was denatured, placed on nitrocellulose filters, and incubated with our cloned cDNA fragment which included the entire pre-pro PTH coding sequence (18).

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0037-9727/89/1913-0233$2.00/0

Nick translation was used to label the cDNA probe with [^{32}P]dCTP (19). The labeled fragment was then hybridized overnight at 43°C, and the filters, after washing, rinsing, and drying were exposed to x-ray film. Densitometry was used to quantitate mRNA.

Cell-Free Transcription. The technique of nuclear runoff (20) was used to determine transcription rate. Individual nuclei were obtained from cells and RNA transcripts were labeled with [^{2}P]UTP by established methods. Nuclear RNA was purified and labeled RNA hybridized to nitrocellulose filters containing plasmids without a cDNA insert or plasmids containing a cDNA insert complementary to the mRNA for pre-pro PTH or that of α-actin (21). After DNA was fixed to the filters, hybridization was carried out for 60 hr at 42°C. After the filters were washed extensively, they were exposed to x-ray film overnight, with the intensity of hybridization being assessed by densitometry.

PTH Measurements. For PTH measurements in short-term incubations, cells were aliquoted into propylene scintillation vials and incubated at 37°C in BME containing desired calcium and 1,25-$(OH)_2D_3$ concentrations. At the end of the incubation, medium was removed and stored at −20°C for radioimmunoassay. For hormone release after culture, medium was removed at the end of the culture period, with cells being rinsed gently in BME containing no added vitamin D or vehicle (3). Fresh medium containing bovine serum albumin (2 mg/ml), desired calcium concentration, and no added 1,25-$(OH)_2D_3$ or vehicle was incubated with the cells at 37°C for 30 min. The medium was then removed and frozen for PTH assay. Assays used for immunoassay were as described (3).

Results

The results described herein have been reported in our previous publications (2–4, 22–24). The observations we have made are as follows:

1. Incubation of bovine parathyroid cells in primary tissue culture with 1,25-$(OH)_2D_3$ at doses varying from 10^{-11} *M* to 10^{-7} *M* caused a decrease in total cytoplasmic pre-pro PTH mRNA levels down to less than 50% of control values at 48 hr (2). These effects were dose responsive, highly specific (no response of control α-actin mRNA), and fully reversible. Initial effects were first noted as early as 6–8 hr but were maximal at 24–48 hr (Fig. 1).

2. The effects of 1,25-$(OH)_2D_3$ on suppressing pre-pro PTH mRNA were greater than 24,25-$(OH)_2D_3$ (about 100-fold) which were greater than 25-$(OH)_2D_3$ (about 100-fold) (2).

3. Nuclear runoff studies showed that the effects of 1,25-$(OH)_2D_3$ on PTH gene expression were due primarily to changes in the transcription rate for mRNA. These effects were noted as early as 1 hr, were dose responsive, and were noted at concentrations as low as 10^{-11} *M* (Fig. 2). They were also reversible and highly specific (4). There was no evidence that 1,25-$(OH)_2D_3$, even at high concentrations, had any toxic effects on cell number or viability or on total RNA or RNA synthesis (2–4).

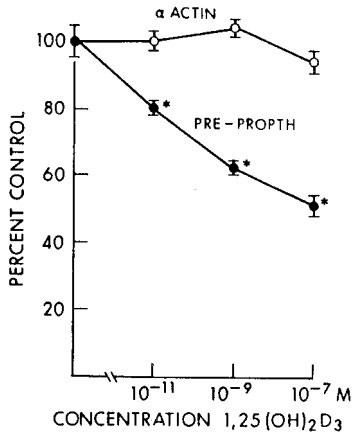

Figure 1. Effects of increasing doses of 1,25-$(OH)_2D_3$ from 10^{-11} to 10^{-7} *M* on levels of pre-pro PTH mRNA at 48 hr in tissue culture compared with control. The blots were also probed with cDNA for α-actin mRNA and showed no changes throughout the experimental period. Results show mean ± SEM of triplicate samples compared with control. Asterisks indicate $P < 0.01$.

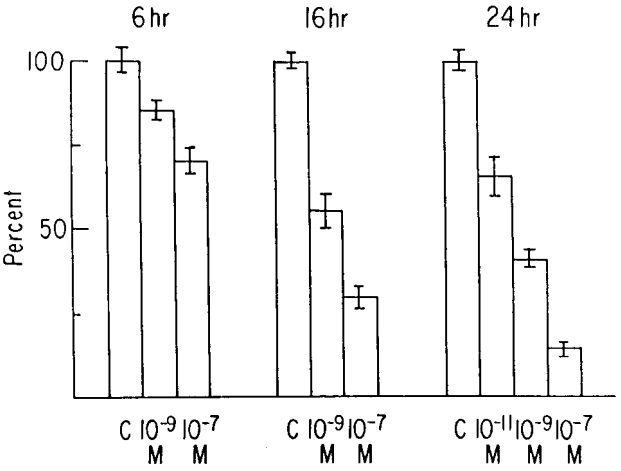

Figure 2. The effects of increasing doses of 1,25-$(OH)_2D_3$ on transcription rates (using nuclear runoff technique) of pre-pro PTH mRNA at doses of 10^{-11}–10^{-7} m*M* at different time periods. Results show mean ± SEM of triplicate samples compared with control, and all decreases were statistically significant ($P < 0.01$).

4. There were no acute effects of 1,25-$(OH)_2D_3$ on PTH release, even at doses as high as 10^{-7} *M*, whereas in cells incubated for longer periods (12–48 hr) with 1,25-$(OH)_2D_3$, there was a decrease in hormone release. This decrease in PTH release was noted at low, normal, and high calcium concentrations compared with control cultures not receiving 1,25-$(OH)_2D_3$ (3). There was a parallel response between the decreases in hormone release and the fall in levels of pre-pro PTH mRNA (3) (Fig. 3). Decreases in hormone release were noted as early as 10–12 hr but were maximal at 48 hr. At concentrations of 10^{-7} *M*, 24,25-$(OH)_2D_3$ produced partial suppression of pre-pro PTH mRNA as well as

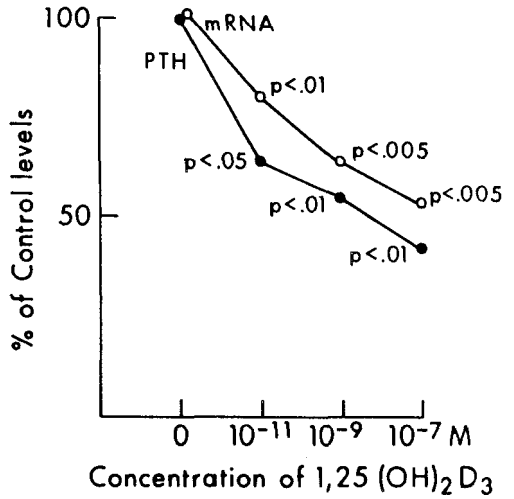

Figure 3. Parallel decreases in levels of pre-pro PTH mRNA and in PTH secretion rates after 48 hr of culture in medium containing increasing doses of 1,25-$(OH)_2D_3$. Values of PTH and mRNA expressed as percentage of vehicle-treated control and probability values indicate level of significance compared with control.

PTH secretion, whereas no effects were noted with 25-$(OH)_2D_3$ or vitamin D_3 (22).

5. The effects of 1,25-$(OH)_2D_3$ in decreasing the transcription of pre-pro PTH mRNA were direct and were not inhibited in cells incubated with cycloheximide at levels totally preventing protein synthesis (23–24). Cells incubated with 1,25-$(OH)_2D_3$ showed a gradual decrease in stored levels of hormone after 8 hr and no effects prior to this point (24).

6. Although we have not examined the half-life of pre-pro PTH mRNA in detail, our preliminary studies (not shown) suggest, like those of Heinrich *et al.* (25), that it is long and of the order of 24–30 hr.

Discussion

These results show that 1,25-$(OH)_2D_3$, the active metabolite of vitamin D_3, has a highly specific, reversible, and physiologic effect on the regulation of PTH gene expression. It appears to interact directly with DNA at the 5′ end of the regulatory portion of the PTH gene without depending on new protein synthesis. Presumably this is through *cis*-acting elements affecting the transcription of pre-pro PTH mRNA. Thus, cells incubated with 1,25-$(OH)_2D_3$ in increasing doses have decreases in total cytoplasmic mRNA levels. Although changes in mRNA half-life could also contribute to the decease in mRNA, these have not yet been demonstrated. In addition to the *in vitro* observations that we have made, there is also *in vivo* data in the rat to support those observations (26).

Our studies have allowed us to integrate a series of molecular observations into a more comprehensive view of parathyroid cell biology. We believe that these results support a biologic model shown in Figure 4. This model suggests that the suppression of PTH gene expression results in a decreased level of pre-pro PTH mRNA and ultimately in hormone synthesis. The parallel fall in mRNA with hormone secretion suggests that the diminution in hormone synthesis ultimately results in decreased secretion from the cell. Our observation that hormone storage initially shows no change but then begins falling after 8 hr is consistent with the proposal that the long half-life of mRNA delays the actual fall in mRNA for some 6 or 8 hr. At this point the fall in mRNA results in decreased hormone synthesis, decreased stores in the cell, and ultimately a diminution of hormone release.

We have also reported that high calcium in the range of 1.7–2.5 m*M in vitro* decreases synthesis of PTH through effects on transcription of the PTH gene (18). These also appear to be specific, dose responsive, and reversible and not dependent on protein synthesis (24). The only difference from the 1,25-$(OH)_2D_3$ effects is our observation that hormone storage increases initially after high calcium exposure to be followed by a decrease after 8 hr. This appears to be due to the fact that high calcium, in addition to suppressing the synthesis of PTH, also impairs secretion. Thus, storage would transiently be increased by the inhibition of hormone release.

The mechanism by which 1,25-$(OH)_2D_3$ and calcium exert their effects on gene expression remains to be explored. Although the mechanism of the calcium effect is more obscure, there is reason to believe that 1,25-$(OH)_2D_3$ behaves like other steroid hormones in its interaction with the hormone receptor which contains both DNA- and ligand-binding domains (10). We have shown that dexamethasone reverses the suppressive effects of 1,25-$(OH)_2D_3$ and not high calcium on PTH gene expression, suggesting that calcium and 1,25-$(OH)_2D_3$ probably act at different sites in the 5′-flanking sequence (27). Transfection studies suggest that the

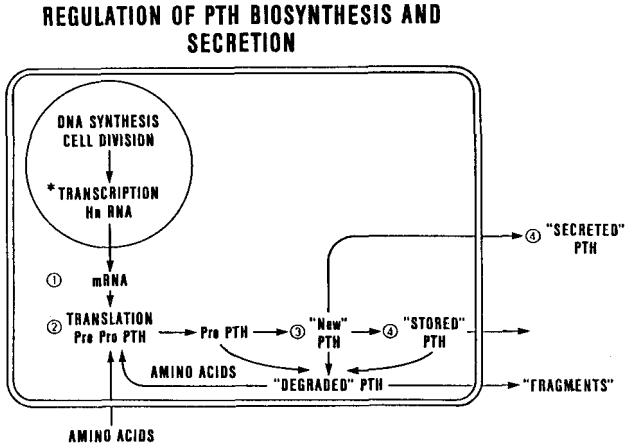

Figure 4. Proposed model for parathyroid cell showing sites of action of 1,25-$(OH)_2D_3$. The asterisk indicates site where 1,25-$(OH)_2D_3$ decreases transcription and results subsequently in (1) decreased steady state levels of pre-pro PTH mRNA, (2) decreased translation of PTH, (3) decreased synthesis of new hormone, and (4) decreased storage and secretion of hormone.

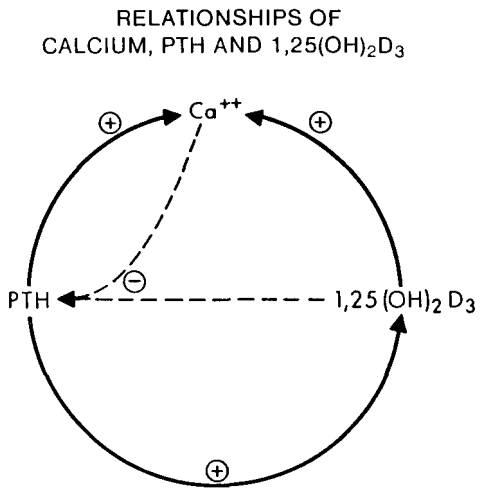

Figure 5. The physiologic loop showing the interrelationships between PTH and 1,25-$(OH)_2D_3$ in the regulation of calcium homeostasis (see Discussion for details).

region of the gene upstream 150 to 250 base pairs from the promoter region of the bovine PTH gene contains the site necessary for 1,25-$(OH)_2D_3$ vitamin D receptor interaction (28).

Figure 5 outlines the physiologic relationships which appear to be present between PTH and 1,25-$(OH)_2D_3$ in their regulation of calcium homeostasis. PTH raises the plasma calcium through its effects on bone and the kidney, while 1,25-$(OH)_2D_3$ produces similar effects by working in concert with PTH on bone and enhancing calcium absorption by the gastrointestinal tract. Thus, both hormones are positive contributors to the plasma calcium. The plasma calcium feeds back to decrease the secretion of PTH, and as we have described, to impair PTH synthesis. It has been known that PTH enhances the synthesis of 1,25-$(OH)_2D_3$ by enhancing 1α-hydroxylase activity, and our findings now clearly show that 1,25-$(OH)_2D_3$ decreases the synthesis and ultimately the secretion of PTH by impairing PTH gene expression. Further studies *in vitro* and in *in vivo* animal models will clarify further the details and mechanism of the 1,25-$(OH)_2D_3$-parathyroid cell interaction. Integration of the effects of 1,25-$(OH)_2D_3$ and PTH are not only critical to normal physiology but also essential to understanding the complexities of the pathophysiology of chronic renal failure and other disorders of mineral metabolism (29, 30).

This work was supported in part by Grants DK 28556 and DK 34822 from the United States Public Health Service.

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