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Predominant role of 25OHD in the negative regulation of PTH expression: Clinical relevance for hypovitaminosis D

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Abstract

Although severe deficiency of bioactive vitamin D (1,25OH2D) causes rickets, mild insufficiency of the hormone, known as hypovitaminosis D, is responsible for the occurrence of secondary hyperparathyroidism and osteoporosis. To clarify the pathophysiology of the disease, we studied the negative feedback effect of 1,25OH2D and its precursor 25OHD on the transcriptional activity of parathyroid hormone (PTH) gene using the PT-r parathyroid cell line. We found that PT-r cells express endogenous 1α-hydroxylase as well as PTH mRNAs. We also found the potent suppressive effect of physiological concentration of 25OHD on the transcriptional activity of PTH gene. A similar effect was obtained with 1,25OH2D but only with pharmacological concentration. Interestingly, the effect of 25OHD was completely abolished when the cells were treated with 1α-hydroxylase inhibitor ketoconazole. These results suggest that the negative feedback regulation of vitamin D on PTH gene transcription occurs not by the end-product 1,25OH2D but by its prohormone 25OHD via intracellular activation by 1α-hydroxylase within the parathyroid cells.

Keywords: Parathyroid hormone; Vitamin D; Hypovitaminosis D; Secondary hyperparathyroidism; Osteoporosis

Introduction

Vitamin D plays an indispensable role in the regulation of calcium and bone metabolism (Jones et al., 1998; Holick, 2007). Provitamin D, synthesized in the skin, is hydroxylated to 25OHD in the liver and then converted to the active form 1,25OH2D by 1α-hydroxylase (1α-OHase) in the kidney. 1,25OH2D enhances the absorption of calcium and phosphate in the epithelium of the small intestine via its specific nuclear receptor (vitamin D receptor; VDR). The genetic defects in either synthesis or action of the hormone may result in severe metabolic bone diseases such as osteomalacia and rickets, as well as hypocalcemia and hypophosphatemia.

Recently, a new form of vitamin D-related disorder has been established, named hypovitaminosis D (Thomas et al., 1998). This occurs in patients with an insufficiency but not a deficiency of vitamin D, in whom the plasma level of 25OHD is relatively low but still within normal limits (>10 ng/ml) and that of 1,25OH2D is normal or even high (Parfitt, 1998). Nevertheless, these patients exhibit elevated plasma PTH, high turnover-type osteoporosis, and an increase in the risk of fracture (Lips, 2001; Lips et al., 2001; Barone et al., 2007). This disorder is frequently recognized in winter and in the elderly population, probably because of the decreased chance of sunlight exposure. However, it is not clarified why the deficiency of the prohormone 25OHD but not bioactive 1,25OH2D elicits secondary hyperparathyroidism with elevated PTH secretion in hypovitaminosis D.

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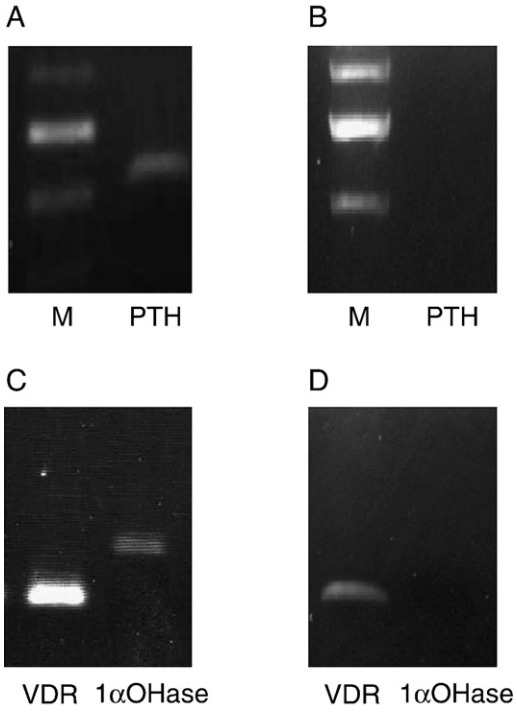

Fig. 1. RT-PCR analysis of the expression of endogenous PTH and 1α-OHase mRNAs in PT-r cells and CA77 cells. The figures show photographs of the ethidium bromide-stained products using agarose gel electrophoresis. cDNA produced from an RT reaction using total RNA from PT-r cells was amplified by PCR with pairs of oligonucleotide primers specific for each mRNA. Bands corresponding to the mRNAs for PTH and 1α-OHase genes were obtained in PT-r (A,C), but not CA77 (B,D) cells, whereas that for VDR was recognized in both cell lines (C,D). No band was amplified in the same reaction without reverse transcriptase (data not shown).

Vitamin D is known to exert a negative effect on PTH synthesis through negative feedback regulation. Indeed, 1,25OH2D has been shown to inhibit PTH gene expression at the transcriptional level (Okazaki et al., 1988; Mackey et al., 1996; Darwish and DeLuca, 1996; Liu et al., 1996; Russell et al., 1999; He et al., 2002; Koszewski et al., 2004; Alimov et al., 2004; Alimov et al., 2005; Jaaskelainen et al., 2005; Kim et al., 2007). One drawback of these *in vitro* studies is that all the examinations were conducted using heterologous cells because of the unavailability of a parathyroid gland-derived homologous cell line, and the precise mode of PTH gene repression by vitamin D in parathyroid cells is still not completely elucidated.

We recently re-characterized the cell line PT-r, which was established from the rat parathyroid gland by Sakaguchi and Aurbach (Sakaguchi et al., 1987), and found that the cells endogenously express PTH mRNA, suggesting the nature of the parathyroid gland origin. We also found the expression of 1α-OHase, which converts the prohormone 25OHD to the bioactive form 1,25OH2D, in this cell line. Since recently 1α-OHase has been shown to be expressed in the human parathyroid gland (Segersten et al., 2002), in this study, we examine the effect of 25OHD as well as 1,25OH2D on PTH gene transcription using the homologous PT-r cells, based on the hypothesis that 25OHD exerts a negative effect on PTH expression via intracellular activation *in situ* by 1α-OHase.

Materials and methods

Reagents

25OHD, 1,25OH2D, and ketoconazole were purchased from Sigma (St. Louis, MO).

Plasmid construction

The 5′-promoter region of the human PTH gene (−2000/+54) was cloned by PCR, and then a variety of deleted/mutated promoter constructs were made and subcloned into a pA3Luc luciferase reporter plasmid using standard molecular biology techniques. The VDREx3-Luc reporter plasmid was a kind gift from Drs. Shigeaki Kato and Ryo Fujiki (Kato et al., 2004).

Reverse transcription–polymerase chain reaction (RT-PCR)

Total RNA was isolated from the PT-r rat parathyroid cell line or the CA77 rat thyroid C cell line using RNeasy RNA extraction kit (Qiagen, Valencia, CA), and 5 μg each of the total RNA was used for the reverse transcription reaction with MMLV reverse transcriptase (Superscript III; Invitrogen, Carlsbad, CA). The cDNA samples obtained was then amplified by Taq DNA

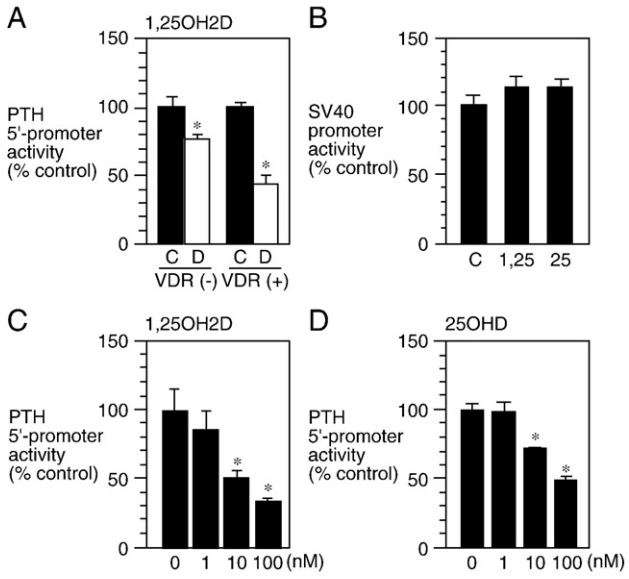

Fig. 2. Inhibitory effects of vitamin D-related metabolites on PTH gene expression in PT-r cells. A. PT-r cells were transfected transiently with PTH_{2000}-Luc without or with VDR expression vector [PTH_{2000}-Luc (μg): RSV-VDR (μg)=1:1], and then treated with 1,25OH2D (10 nM) for 48 h. 1,25OH2D significantly inhibited the transcriptional activity of PTH gene in both conditions, but the effect was more obvious when VDR was co-expressed. B. PT-r cells were transfected transiently with SV40-Luc with VDR expression vector [SV40-Luc (μg): RSV-VDR (μg)= 1:1], and then treated with 1,25OH2D for 48 h. No suppressive effect was observed, indicating the promoter-specific effect of 1,25OH2D. C, D. PT-r cells were transfected transiently with PTH_{2000}-Luc with VDR expression vector [PTH_{2000}-Luc (μg): RSV-VDR (μg)=1:1], and then treated with various doses of 1,25OH2D (0–100 nM) (C) or 25OHD (0–100 nM) (D) for 48 h. Significant inhibitory effects were observed at or above 10 nM in both 1,25OH2D and 25OHD, although the degree of inhibition was more potent in the former. *$p<0.05$ vs. corresponding control.