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Distribution and regulation of the 25-hydroxyvitamin D_3 1α-hydroxylase in human parathyroid glands

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ABSTRACT

Parathyroid glands express the 25-hydroxyvitamin D_3 1α-hydroxylase (1αOHase). 1,25-dihydroxyvitamin D_3 (calcitriol) synthesized by extrarenal tissues generally does not enter the circulation, but plays an autocrine/paracrine role specific to the cell type, and is regulated by the needs of that particular cell. While the role of calcitriol produced in the parathyroid glands presumably is to suppress PTH and cell growth, its regulation in this cell type has not been defined. In the present study, we found that regulation of the human parathyroid 1αOHase differs from the renal enzyme in that it is induced by FGF-23 and extracellular calcium. Hyperplastic parathyroid glands from patients with chronic kidney failure normally display a heterogeneous cellularity. We found that the 1αOHase is expressed at much higher levels in oxyphil cells than in chief cells in these patients. Recent findings indicate that oxyphil cell content is increased by treatment with calcium receptor activators (calcimimetics). Here, we demonstrate that the calcimimetic cinacalcet increases the expression of 1αOHase in human parathyroid cultures. Additionally, we found that the 1αOHase in human parathyroid cultures is functionally active, as evidenced by the ability of the enzyme to 1-hydroxylate 25(OH)D_3 in parathyroid monolayers. Calcium, as well as cinacalcet, also induced expression of the degradation enzyme 24-hydroxylase, indicating the presence of a negative feedback system in the parathyroid cells. Therefore, local production of 1αOHase suggests an autocrine/paracrine role in regulating parathyroid function and may mediate, in part, the suppression of PTH by calcium and FGF-23.

1. Introduction

Vitamin D_3 is a prohormone that is activated by two metabolic steps [1]. The first, 25-hydroxylation, occurs mainly in the liver and is relatively unregulated, so that 25-hydroxyvitamin D_3 [25(OH)D_3] is the major circulating metabolite of vitamin D. The second step is 1-hydroxylation to form the vitamin D hormone, 1,25-dihydroxyvitamin D_3 (calcitriol). The latter reaction is carried out by the 25-hydroxyvitamin D-1α-hydroxylase (1αOHase; CYP27B1), and is highly regulated, primarily at the level of expression of the enzyme. The physiological actions of calcitriol encompass both classical and non-classical functions. In the classical endocrine role, circulating calcitriol produced by the kidney regulates systemic calcium and phosphate homeostasis through actions on the intestine, bone, kidney and parathyroid gland [1]. In the non-classical autocrine/paracrine role, local production of calcitriol in non-renal tissue carries out specialized non-calcitropic functions, such as induction of cell differentiation, inhibition of cell growth, immunomodulation and control of other hormonal systems [1,2].

One of the extrarenal tissues that produce calcitriol is the parathyroid gland, which plays a central role in calcium and phosphate homeostasis through feedback loops involving calcium, phosphate and vitamin D. Segersten et al. first reported that human parathyroid glands express 1αOHase protein and mRNA, and that the enzyme was more highly expressed in hyperplastic glands from patients with primary and secondary hyperparathyroidism compared to normal glands [3]. Our laboratory subsequently demonstrated the presence of 1αOHase in cultured bovine parathyroid cells, and showed that the enzyme was functionally active, converting 25(OH)D_3 to 1-hydroxylated metabolites, and inducing the major enzyme involved in the degradation of calcitriol, 25-hydroxyvitamin D_3 24-hydroxylase (24-OHase, CYP24A1) [4]. The vitamin D receptor (VDR) is also highly expressed in parathyroid

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cells, making the gland a well-established target tissue for vitamin D action [5,6]. The expression in the parathyroid gland of the VDR, as well as the enzymes responsible for activation and degradation of vitamin D, suggests an autocrine/paracrine function for locally produced calcitriol.

Circulating levels of calcitriol produced by the kidney are regulated by the need for calcium and phosphate. Calcium may have a direct suppressive effect on the renal 1αOHase [7], but most of the effect of calcium is mediated by parathyroid hormone (PTH) [8,9] via cyclic AMP [10]. Phosphate also negatively regulates the renal 1αOHase [11], mainly via fibroblast growth factor 23 (FGF-23), a phosphaturic hormone involved in phosphate homeostasis and skeletogenesis [12,13]. While the renal 1αOHase is regulated by the need for calcium and phosphate, the extrarenal 1αOHase is regulated by the specific needs of the individual cell type [14]. At present, little is known about the regulation of the 1αOHase in the parathyroid gland.

Parathyroid glands from patients with secondary hyperparathyroidism are heterogeneous, being comprised of chief, oxyphil, transitional, and water-clear cells. In addition, the glands are often a mixture of nodular and diffuse hyperplastic tissue. Differential expression of 1αOHase in the various cells types in hyperplastic parathyroid tissue has not been reported. Here, we examined the expression and distribution of the 1α-OHase in hyperplastic human parathyroid tissue, and regulation of the enzyme in cultured human parathyroid cells.

2. Materials and methods

2.1. Human parathyroid cultures

Parathyroid tissue was obtained from patients undergoing parathyroidectomy due to uremic secondary hyperparathyroidism. The study was approved by the Human Research Protection Office of Washington University School of Medicine; informed consent was obtained for collection of the tissue. The tissue was dispersed with collagenase as previously described [15]. Briefly, parathyroid tissue was finely minced and placed in a mixture of DME:Ham's F-12 medium (50:50) containing 0.5 mM calcium and collagenase (3000 U/ml of collagenase XI-S; Sigma–Aldrich; Saint Louis, MO, USA). The suspension (10 ml media/g tissue) was agitated in a shaking water bath at 37 °C for 90–120 min. Periodic passage of the mixture through the tip of a 10-ml pipette assisted in the disaggregation. The digested tissue was washed with serum-free culture medium containing DME:Ham's F-12 (50:50), 1 mM $CaCl_2$, 15 mM HEPES, 100 IU/ml penicillin, 100 μg/ml streptomycin, 5 μg/ml insulin, 2 mM glutamine, 1% nonessential amino acids, 5 μg/ml holo-transferrin, 0.1% bovine serum albumin (fraction V). At this stage, cells were either plated for experimentation, or frozen for later use. For monolayers (calcium and calcimimetic studies), cells were plated in 24-well plates at a concentration of approximately 200,000 cells/well in the above medium containing 10% fetal bovine serum to allow for cell attachment. After 24 h, the medium was replaced with serum-free culture medium. This serum-free medium is used routinely in our parathyroid cultures. It suppresses growth of fibroblasts, and is preferred for studies involving vitamin D compounds, since serum can contribute undesired amounts of vitamin D; in addition, serum free media is often used to avoid unwanted DBP which may bind vitamin D and its metabolites, and interfere with the uptake of vitamin D and its metabolites into the cells. Monolayers were generally processed by day 4 in culture. For cAMP and FGF-23 experiments, we found that culturing parathyroid cells in suspension produced more consistent results, and were preferable to cells cultured as monolayers. To culture cells in this manner, dispersed cells were placed in 24-well plates in serum-free medium. The absence of serum prevents attachment of the cells to the well, keeping the cells in suspension. Under these conditions, the parathyroid cells will clump and form multiple small, irregularly shaped organoids. The cells grown in suspension were used within 2 days in culture.

2.2. Immunoblot and immunohistochemistry analysis

A rabbit polyclonal antibody to 1αOHase was raised commercially (Open Biosystems, Huntsville, AL, USA) against a synthetic peptide based on the last 14 C-terminal amino acids of the rat CYP27B1 (Accession No. AF000139). For immunoblot analysis, a kidney from a vitamin D deficient rat and human parathyroid tissue from a patient with primary hyperparathyroidism were homogenized in a buffer consisting of 150 mM NaCl, 1.0% Triton X-100, 0.5% Na-deoxycholate, 0.1% sodium dodecyl sulfate and 50 mM Tris (pH 8.0), containing a commercial peptidase inhibitor (cOmplete™, Roche Diagnostics, Indianapolis, IN, USA). Samples were resolved on 12% PAGE gels and transferred to PDVF membranes. The membranes were blocked with PBS containing 0.1% Tween 20 and 5% nonfat dry milk at room temperature for 1 h, and then incubated overnight in blocking buffer containing a 1:500 dilution of the 1αOHase antibody with or without 4 μg/ml of 1αOHase peptide. Rabbit serum obtained pre-immunization (1:500 dilution) served as a negative control. After washing, membranes were incubated for 1 h at room temperature with goat anti-rabbit secondary antibody conjugated to horseradish peroxidase (Sigma–Aldrich) at a 1:50,000 dilution. Enhanced chemiluminescent (ECL) reagent from GE HealthCare (Piscataway, NJ, USA) was used for immunodetection.

Immunohistochemical staining of 1αOHase protein was performed on formalin-fixed, paraffin-embedded human parathyroid glands tissue using the 1αOHase antiserum and HRP-conjugated anti-rabbit secondary antibody (ImmPRESS™ Reagent, Vector Laboratories, Burlingame, CA, USA). The tissue was deparaffinized, rehydrated, and microwaved at high intensity for 8 min in 10 mM citric acid, pH 6.0, and allowed to cool for 10 min. The slides were washed with PBS and blocked with 2.5% pre-immune horse serum (Vector Laboratories, Inc., Burlingame, CA, USA). The sections were incubated overnight at 4 °C with a 1:370 dilution of 1αOHase antibody with or without 4 μg/ml of 1αOHase peptide. Rabbit serum served as the negative control. The HRP-conjugated second antibody was applied for 30 min at room temperature, and the immune complexes visualized with 3-amino-9-ethylcarbazole (AEC) substrate-chromagen (Invitrogen-Life Technologies, Grand Island, NY, USA).

Immunostaining of 1αOHase was quantified in hyperplastic parathyroid glands from 13 patients, as previously described [16]. Briefly, 200× images of stained tissue sections were captured, converted to gray scale, and analyzed using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA). The intensity of staining was quantified using the optical density function of the software. The average integrated optical density (IOD) was calculated by dividing the sum IOD by the sum area. The IOD/area of a tissue section immunostained using negative control rabbit serum was subtracted from IOD/area for the 1OHase antibody sections. The corrected IOD/area for the chief and oxyphil cells calculated and results reported as % chief cell average.

2.3. Real-time PCR

Total RNA was isolated using RNAzol Bee (Tel-Test, Friendswood, TX, USA) per manufacturer's instructions. Reverse transcription of the RNA was carried out using oligo-dT primer and SMART MMLV reverse transcriptase (Clontech Laboratories, Mountain View, CA, USA). Real-time PCR (qPCR) was