The vitamin D receptor in the proximal renal tubule is a key regulator of serum 1α,25-dihydroxyvitamin D₃

Yongji Wang,1,2 Jinge Zhu,1 and Hector F. DeLuca1

1Department of Biochemistry, University of Wisconsin, Madison, Wisconsin; and 2Vitamin D Research Institute, Shaanxi University of Technology, Shaanxi Province, China

Submitted 9 September 2014; accepted in final form 21 November 2014

Wang Y, Zhu J, DeLuca HF. The vitamin D receptor in the proximal renal tubule is a key regulator of serum 1α,25-dihydroxyvitamin D₃, Am J Physiol Endocrinol Metab 308: E201–E205, 2015. First published November 25, 2014; doi:10.1152/ajpendo.00422.2014.—It is well established that the mitochondria of proximal convoluted tubule cells of the kidney are the site of production of 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]. The production of 1,25(OH)₂D₃ at this site is tightly regulated. Parathyroid hormone markedly stimulates 1,25(OH)₂D₃ production, whereas 1,25(OH)₂D₃ itself suppresses production. The mechanism of suppression by 1,25(OH)₂D₃ has not yet been elucidated. We have now found that in the absence of vitamin D (vitamin D deficiency), the vitamin D receptor (VDR) is found in the interior of the apical brush border of the proximal tubule cells. This is unique for the proximal tubule cells, since this has not been observed in the distal tubule cells or in other epithelial cells, such as intestinal mucosa. Administration of 1,25(OH)₂D₃ to vitamin D-deficient rats results in the movement of VDR from the brush border to the cytoplasm and nucleus presumably bound to reabsorbed 1,25(OH)₂D₃. The VDR bound to 1,25(OH)₂D₃ suppresses expression of 25-hydroxyvitamin D₃ 1α-hydroxylase and stimulates the 25-hydroxyvitamin D₃ 24-hydroxylase. Thus, VDR in the apical brush border of the proximal convoluted tubule cells serves to “sense” the level of circulating 1,25(OH)₂D₃ and modulates the activity of the 1α-hydroxylase and the 24-hydroxylase accordingly.

1,25-dihydroxyvitamin D; proximal renal tubule; vitamin D receptor; 24-hydroxylase; 1α-hydroxylase; calcium

THE KIDNEY IS THE MAJOR ORGAN for the regulation of serum 1α,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃]. The production and degradation of 1,25(OH)₂D₃ at this site is tightly regulated in a complex fashion. The 1α-hydroxylase (CYP27B1) is clearly located in proximal convoluted tubule cells and is largely responsible for circulating 1,25(OH)₂D₃ (4, 7, 14). The principle enzyme that degrades 1,25(OH)₂D₃ is the 24-hydroxylase (CYP24A1). The CYP24A1 is not expressed in vitamin D-deficient animals and is induced by 1,25(OH)₂D₃ bound to the vitamin D receptor (VDR) (14, 18), representing a major regulatory factor (5). In addition, 1,25(OH)₂D₃ itself can suppress CYP27B1, but this also requires VDR (7, 14). In our examination of the VDR by immunohistochemistry, we were struck by the unique location of VDR in the brush borders of the proximal renal tubule cell of vitamin D-deficient rats. When 1,25(OH)₂D₃ is administered, the VDR leaves the brush border and relocates to the cytoplasm and eventually to the nucleus. This results in the suppression of CYP27B1 and the induction of CYP24A1, thereby regulating serum levels of 1,25(OH)₂D₃. We suggest that the brush border-associated VDR serves as a sensor of 1,25(OH)₂D₃ in the filtered plasma. If 1,25(OH)₂D₃ is low or VDR is absent from the nucleus, the CYP24A1 is not induced and the CYP27B1 is not suppressed, resulting in very high levels of 1,25(OH)₂D₃ in blood (20). Previously, we demonstrated that the VDR is low or undetectable in kidney under hypocalcemic conditions (10), preventing suppression of CYP27B1 by reabsorbed 1,25(OH)₂D₃ and induction of CYP24A1. This article presents the concept that VDR in the proximal tubule brush border serves to sense circulating 1,25(OH)₂D₃ and is the mechanism whereby CYP27B1 and CYP24A1 are regulated, which in turn plays a major role in regulating serum calcium.

MATERIALS AND METHODS

Antibodies. The antibodies were used as described previously (22). Animals. All animals were managed in accordance with University of Wisconsin standards and protocols for animal care and use, and our experiments were approved by the College of Agricultural and Life Sciences Animal Care and Use Committee. Male weanling rats (Sprague-Dawley strain) used in this study were from vitamin D-deficient mothers. For the vitamin D-deficient studies, the rats weighing ~50 g were housed in a room devoid of ultraviolet light, preventing the endogenous production of vitamin D₃. These animals were maintained on a synthetic vitamin D-deficient diet (17) containing either 0.47 or 1.2% calcium for the 1st week and then fed the same diet but containing 0.02% calcium for an additional 3 wk. One week before the start of the experiment, they were returned to the 1.2% calcium diet. The rats were then randomly grouped and maintained on the same diet. All diets contained 0.3% phosphorus. Vitamin D-replete rats were given 25 IU vitamin D₃ three times/wk, as described previously (3, 15), and fed the 0.47% calcium diet. Where indicated, rats were given four intraperitoneal injections of 780 pmol (324 ng) of 1,25(OH)₂D₃ (SAFC, Madison, WI) in 0.1 ml of propylene glycol. Three doses were given at 24-h intervals and the last dose 12 h before euthanization. The control groups received vehicle.

Measurement of serum calcium and 1,25(OH)₂D₃. Serum calcium determinations were carried out by atomic absorption using Model 3110 (Perkin-Elmer, Norwalk, CT). The procedure for the 1,25(OH)₂D₃ assay was modified from a method designed in our laboratory (1).

Tissue collections. Blood was collected, allowed to clot, and then centrifuged at 3,000 g for 15 min, followed by a second spin at 17,000 g for 1 min to yield serum. All tissues were immediately fixed with cold 3.7% paraformaldehyde after removal and processed as described previously (22).

Quantitative RT-PCR. Total RNA was isolated from mouse kidney with Tri reagent (Molecular Research Center, Cincinnati, OH) according to the manufacturer’s protocol. Quantitative PCR (qPCR) was performed as described previously (23).

Immunohistochemistry. Immunohistochemical staining was performed as described previously (22).


RESULTS

Serum levels of calcium and 1,25(OH)\textsubscript{2}D\textsubscript{3} in vitamin D-deficient and normal rats. Vitamin D-deficient (−D) rats on a 0.47% calcium diet had low levels of serum calcium at 5.9 mg/dl (Fig. 1A). Administration of four daily doses of 1,25(OH)\textsubscript{2}D\textsubscript{3} at 324 ng/dose raised serum calcium to 10.6 mg/dl (P < 0.01). When the same dose of 1,25(OH)\textsubscript{2}D\textsubscript{3} was administered to normal animals sufficient in vitamin D (+D) on the same 0.47% calcium diet, hypercalcemia (12.1 mg/dl) resulted (P < 0.05).

Serum 1,25(OH)\textsubscript{2}D\textsubscript{3} was low but detectable in normal rats, whereas it was undetectable in vehicle-treated vitamin D-deficient animals (Fig. 1B). In normal animals, there was a slight elevation of serum 1,25(OH)\textsubscript{2}D\textsubscript{3} in the +D/1,25(OH)\textsubscript{2}D\textsubscript{3} rats (25.2 pg/ml) compared with +D/rats given vehicle (13.8 pg/ml) (P = 0.2446). The increase of serum 1,25(OH)\textsubscript{2}D\textsubscript{3} concentration was greater when 1,25(OH)\textsubscript{2}D\textsubscript{3} was administered to −D animals (30.4 pg/ml). These data confirm earlier studies done with radiolabeled vitamin D (3, 21).

1,25(OH)\textsubscript{2}D\textsubscript{3} increases the amount and causes a relocation of VDR from cytoplasm to nucleus in the proximal renal tubular cells from normal rats. Immunohistochemical detection of VDR (22) was carried out in the kidney of +D/vehicle animals. A significant amount of VDR staining was found in the cytoplasm of the proximal tubular cells (Fig. 2, C and D). In particular, the VDR staining was also observed in the interior of the proximal tubular brush border identified by villin, a molecular marker for the brush border. The nuclear staining was present but low. In contrast, the receptor was clearly present in high levels in the nuclei of the distal tubules, as described previously (22). Villin was not detected in the distal tubules. In the +D/1,25(OH)\textsubscript{2}D\textsubscript{3} animals, no significant VDR staining was found in cytoplasm of the renal tubular cells (Fig. 2, E and F).
The brush border-associated VDR is found in proximal renal tubular cells in vitamin D-deficient rats, and 1,25(OH)2D3 causes relocation of the VDR from the brush border to cytoplasm. In vitamin D-deficient rats, VDR was absent from nuclei but localized in the interior of the brush border membrane, determined by colocalization with villin (Fig. 3, A and B). When 1,25(OH)2D3 was administered to −D rats, VDR was detected mainly in the cytoplasm of the proximal tubular cells (Fig. 3, C and D). More importantly, VDR was low or undetectable in nuclei of the proximal tubule cells of these animals. In contrast to the proximal tubules, VDR was localized in the nuclei of the distal tubular cells in both vitamin D-deficient animals and animals given 1,25(OH)2D3 (Fig. 3).

The nuclear location of VDR is associated with 1,25(OH)2D3-induced transcription of target genes. VDR transcripts were low in kidneys of vitamin D-deficient animals. There was a 2.6-fold increase (P < 0.01) in VDR mRNA in response to 1,25(OH)2D3 in the +D animals (Fig. 4A). Vitamin D deficiency increased CYP27B1 transcripts (Fig. 4, B and C). This result is consistent with previous reports on the increase of 1α-hydroxylase transcription by vitamin D deficiency and calcium restriction (10, 12). Administration of 1,25(OH)2D3 to both +D and −D rats decreased CYP27B1 mRNA below controls (P < 0.01 for both +D and −D rats).

CYP24A1 is the most 1,25(OH)2D3-responsive gene known, and its transcription is VDR dependent (5). The transcription of CYP24A1 occurs predominantly in the proximal tubules (13). Thus, the CYP24A1 mRNA level demonstrates the VDR-dependent transcriptional activity in proximal tubules. As expected, vehicle-treated vitamin D-deficient animals had extremely low levels of CYP24A1 mRNA in response to 1,25(OH)2D3 administration of 1,25(OH)2D3 had a significant increase in the production of CYP24A1 mRNA in response to 1,25(OH)2D3 (Fig. 4).

In vitamin D-deficient rats, VDR was absent from nuclei but localized in the interior of the brush border membrane, determined by colocalization with villin (Fig. 3, A and B). When 1,25(OH)2D3 was administered to −D rats, VDR was detected mainly in the cytoplasm of the proximal tubular cells (Fig. 3, C and D). More importantly, VDR was low or undetectable in nuclei of the proximal tubule cells of these animals. In contrast to the proximal tubules, VDR was localized in the nuclei of the distal tubular cells in both vitamin D-deficient animals and animals given 1,25(OH)2D3 (Fig. 3).
14, 19). 1,25(OH)2D3 binds VDR, which then induces the biologically inactive metabolite calcitroic acid (2, 5, 8). The enzyme, CYP24A1, inactivates 1,25(OH)2D3 by converting it to 24,25(OH)2D3. The receptor was selectively found in the parathyroid gland (PG). The mouse IgG control did not have any significant staining (data not shown). Note that VDR is found mainly in the nuclei of epithelial cells from intestine, parathyroid, and skin. Scale bar in the fluorescent images, 100 μm; n = 5–6 animals.

Calcium transporters and binding proteins such as CaBP-D28k are expressed exclusively in distal renal tubules and collecting ducts (22). In vitamin D-deficient and normal rats, 1,25(OH)2D3 dramatically induced CaBP-D28k gene transcription (P < 0.01 for both −D and +D rats; Fig. 4E).

The nucleus-cytosol relocation of VDR is not found in intestinal, parathyroid, or skin tissues. Under the condition of vitamin D deficiency, VDR is expressed in epithelial cells of intestine, skin, and parathyroid glands and is largely in the nuclei (Fig. 5). However, specifically in the proximal tubule cells, VDR is localized in the brush borders in the absence of 1,25-(OH)2D3 (vitamin D deficiency) and moves to cytoplasm and ultimately to the nucleus following the administration of vitamin D.

DISCUSSION

The proximal tubule cells of the kidney serve as the endocrine organ that converts 25-hydroxyvitamin D3 into 1,25(OH)2D3. This conversion is carried out by the enzyme CYP27B1. Another enzyme, CYP24A1, inactivates 1,25(OH)2D3 by converting it to the biologically inactive metabolite calcitroic acid (2, 5, 8, 14, 19). 1,25(OH)2D3 binds VDR, which then induces CYP24A1. At the same time, 1,25(OH)2D3 bound to VDR suppresses CYP27B1, resulting in a reduction of circulating 1,25(OH)2D3. This regulation is superimposed on the well-known parathyroid hormone (PTH) stimulation of CYP27B1 and the suppression of CYP24A1 (9, 11, 16). Under hypocalcemic conditions, expression of VDR is markedly reduced in the kidney (10). When VDR is low, reabsorbed 1,25(OH)2D3 is unable to suppress CYP27B1 and/or activate CYP24A1. This results in high levels of circulating 1,25(OH)2D3 (19, 20). This occurs when animals are fed a low-calcium diet, resulting in circulating levels of 1,25(OH)2D3 as high as 750 pg/ml in serum (20). The exact mechanism whereby a lack of dietary calcium and hence hypocalcemia causes the downregulation of VDR is yet unknown. Thus, hypocalcemia causes an unbridled production of 1,25(OH)2D3 via large elevations of PTH in an attempt to correct hypocalcemia. The elevated PTH stimulates CYP27B1, whereas 1,25(OH)2D3 is unable to stimulate CYP24A1 or suppress CYP27B1 because of a lack of VDR. Therefore, VDR in the proximal tubule cells serves as an important regulator of 1,25(OH)2D3 production. We postulate that the VDR at the apical membrane serves to sense the level of 1,25(OH)2D3 in the circulation, because as it binds the filtered 1,25(OH)2D3, it acts as an inhibitor of CYP27B1 (Fig. 6). If calcium deficiency exists, VDR is undetectable (10) or absent in the nucleus. Consequently, CYP27B1 is not inhibited and CYP24A1 is not induced, giving rise to high levels of plasma 1,25(OH)2D3. Thus, the VDR in the proximal tubule cells plays an important role in the regulation of metabolism of 1,25(OH)2D3 and in the kidney.

ACKNOWLEDGMENTS

We sincerely thank Dr. Lori Plum for discussing the experimental design and results, Xiaohong Ma for maintaining the animals, and Caroline M. Schriebel for qPCR analysis of gene transcription. We also thank Laboratory Manager Lance Rodenkirch in the W. M. Keck Laboratory for Biological

Fig. 5. The nucleus-cytosol relocation of VDR was not found in the intestinal (A), skin (B), or parathyroid (C and D) tissues from the −D animals on a 0.02% (−C) or 1.2% (−D) calcium diet. A: colocalization of VDR (red), E-cadherin (green), and DAPI (blue) in the duodenal tissue sections. B: colocalization of VDR (red) and DAPI (blue) in the skin tissue sections. C and D: colocalization of VDR (red) and DAPI (blue) in the thyroid gland sections. The receptor was selectively found in the parathyroid gland (PG). The mouse IgG control did not have any significant staining (data not shown). Note that VDR is found mainly in the nuclei of epithelial cells from intestine, parathyroid, and skin. Scale bar in the fluorescent images, 100 μm; n = 5–6 animals.

Fig. 6. Diagrams illustrating the special mechanism that allows the proximal renal tubular cells to sense the circulating 1,25(OH)2D3 and regulate its production. Vitamin D deficiency (−D; top) causes the VDR (6-sided purple arrowsheads) to remain at the brush border in the proximal tubules. Administration of 1,25(OH)2D3 (red triangles) results in movement of the receptor from the brush border to cytoplasm and eventually to the nucleus, inhibiting expression of the 1α-hydroxylase and inducing the 24-hydroxylase. Under conditions of vitamin D sufficiency (+D; bottom), VDR is located in the cytoplasm and nuclei. Administration of 1,25(OH)2D3 to the +D animals causes profound movement to the nucleus, inhibiting expression of the 1α-hydroxylase and inducing the 24-hydroxylase, resulting in a reduction of the circulating 1,25(OH)2D3. We postulate that the VDR at the apical membrane of the PT serves to sense the circulating level of 1,25(OH)2D3 and mediates suppression of CYP27B1 and stimulation of CYP24A1.
Images at the University of Wisconsin-Madison for assistance in taking confocal images.

GRANTS
This work was supported by funds provided by the Wisconsin Alumni Research Foundation.

DISCLOSURES
The authors have nothing to disclose.

AUTHOR CONTRIBUTIONS
Y.W. and H.F.D. conception and design of research; Y.W. and J.Z. performed experiments; Y.W., J.Z., and H.F.D. analyzed data; Y.W., J.Z., and H.F.D. interpreted results of experiments; Y.W. prepared figures; Y.W. drafted manuscript; Y.W., J.Z., and H.F.D. edited and revised manuscript; Y.W., J.Z., and H.F.D. approved final version of manuscript.

REFERENCES