Regulation of the murine renal vitamin D receptor by 1,25-dihydroxyvitamin D_3 and calcium

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Renal vitamin D receptor (VDR) is required for 1,25-dihydroxyvitamin D_3-[1,25$(OH)_2D_3$]-induced renal reabsorption of calcium and for 1,25$(OH)_2D_3$-induced 1,25$(OH)_2D_3$ 24-hydroxylase. The long-term effect of vitamin D and dietary calcium on the expression of renal VDR was examined in the nonobese diabetic mouse. Vitamin D-deficient and vitamin D-replete mice were maintained on diets containing 0.02%, 0.25%, 0.47%, and 1.20% calcium with or without 50 ng of 1,25$(OH)_2D_3$ per day. Vitamin D-replete mice on a 1.20% calcium diet had renal VDR levels of 165 fmol/mg protein. Calcium restriction caused renal VDR levels to decrease to <30 fmol/mg protein in vitamin D-deficient mice and to ≈80 fmol/mg protein in vitamin D-replete mice. When dietary calcium was present, 50 ng of 1,25$(OH)_2D_3$ elevated the VDR levels 2- to 10-fold, depending on vitamin D status and the level of calcium. In the absence of either vitamin D or calcium, the VDR mRNA was expressed at a basal level. 1,25$(OH)_2D_3$ supplementation caused relative VDR mRNA to increase 8- to 10-fold in the vitamin D-deficient mouse when dietary calcium was available. This increase was completely absent in the calcium-restricted mice. This *in vivo* study demonstrates that 1,25$(OH)_2D_3$ and calcium are both required for renal VDR mRNA expression above a basal level, furthering our understanding of the complex regulation of renal VDR by 1,25$(OH)_2D_3$ and calcium.

The vitamin D receptor (VDR) is a nuclear protein responsible for mediating the biological actions of vitamin D. 1,25-Dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the functional metabolite of vitamin D, exerts its actions by binding to the VDR and altering the transcriptional rate of target genes. The protein products of these genes then carry out the functions of vitamin D, including the maintenance of serum calcium and phosphorus homeostasis, regulation of cellular proliferation and differentiation, and modulation of the immune system (1).

Studies both *in vivo* and *in vitro* have shown that the biological response to 1,25$(OH)_2D_3$ is directly related to the VDR content of target tissues (2, 3). Thus, understanding the regulation of the VDR is critical to our understanding of the vitamin D endocrine system. The receptor is developmentally regulated, expressed in a tissue-specific manner, and regulated by a variety of physiological factors and hormones, including calcium and 1,25$(OH)_2D_3$ (4).

The mechanism by which calcium and 1,25$(OH)_2D_3$ regulate the VDR is not known. *In vitro* experiments in pig kidney cells (LLC-PK1), mouse fibroblasts (3T6), rat osteosarcoma cells (Ros 17/2.8), and several human osteosarcoma cell lines (MG-63, SaOs-2, and U2-Os) have demonstrated that 1,25$(OH)_2D_3$ up-regulates the VDR at least partially through the activation of gene expression (5–8). Experiments studying the autoregulation of the VDR in LLC-PK1, 3T6, Ros 17/2.8, and rat intestinal epithelial (IEC-6) cells concluded that 1,25$(OH)_2D_3$ increases VDR content primarily through stabilization of the receptor, because little or no increase in VDR mRNA was observed (9–11). The ability of 1,25$(OH)_2D_3$ to increase VDR content *in vivo* was first demonstrated by Costa and Feldman (12), who noted that s.c.-injected 1,25$(OH)_2D_3$ significantly up-regulated renal VDR, while having minimal effect on duodenal VDR. Subsequent experiments have demonstrated that whereas 1,25$(OH)_2D_3$ and calcium do not impact expression of the VDR in the intestine, they do regulate VDR expression in the parathyroid gland and kidney (10, 13–15). In rat kidney, vitamin D_3 or 1,25$(OH)_2D_3$ supplementation has been shown to increase VDR content up to 5-fold, as long as dietary calcium is present (14, 16, 17). Whereas multiple reports have concluded that renal VDR mRNA is not altered by 1,25$(OH)_2D_3$ (10, 13), Brown *et al.* (18) observed a modest induction of VDR mRNA in rats fed a high-calcium diet.

Expression of renal VDR is required for 1,25$(OH)_2D_3$-mediated renal reabsorption of calcium, an important mechanism in the regulation of serum calcium. Additionally, the metabolism of 1,25$(OH)_2D_3$ is influenced by renal VDR expression. This article describes a study on the long-term effects of vitamin D status and dietary calcium on renal VDR expression in mice.

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Materials and Methods

Chemicals. 1,25$(OH)_2D_3$ was acquired from Tetrionics (Madison, WI). 1,25$(OH)_2$[26,27-^{3}H]D_3 was purchased from Perkin–Elmer (Boston).

Mice and Diets. Nonobese diabetic mice were obtained from The Jackson Laboratory. Diabetic mice (serum glucose >300 mg/dl) were excluded from this study, although diabetes incidence did not affect the parameters studied. Breeding pairs were maintained on LabDiet mouse diet 5015 (PMI Nutrition International, St. Louis). For vitamin D-deficiency studies, visibly pregnant females were transferred into separate housing in which all fluorescent lighting was shielded, preventing the endogenous production of vitamin D_3. These females were fed a 1.20% calcium diet lacking vitamin D. Male and female offspring were randomly weaned onto purified diet that contained 0.02%, 0.25%, 0.47%, or 1.20% calcium. This diet was fortified with Wesson oil containing vitamins A, E, and K. For each dietary calcium level, there was a control group that did not receive dietary 1,25$(OH)_2D_3$, and an experimental group that received 50 ng of 1,25$(OH)_2D_3$ daily, based on consumption of 3.5 g per mouse per day. Vitamin D-replete mouse studies were conducted with mice that were housed under fluorescent lighting. The purified diet in these studies [0.02%, 0.25%, 0.47%, and 1.20% calcium with or without 50 ng of 1,25$(OH)_2D_3$] was fortified with oil containing vitamins A, D, E, and K. Mice were maintained on purified diets for 200 days, except for the calcium-restricted, vitamin D-deficient mice, which were killed after 3 weeks on purified diet to prevent death by hypocalcemic tetany. Experimental protocols were reviewed and approved by the Research Animal Resources Center (University of Wisconsin, Madison).

Abbreviations: VDR, vitamin D receptor; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 1α-hydroxylase, 25-hydroxyvitamin D_3-1α-hydroxylase; 24-hydroxylase, 25-hydroxyvitamin D_3-24-hydroxylase.

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Serum Analysis. Mice were bled biweekly and serum calcium was determined by atomic absorption spectrometry. At death, serum was isolated and $1,25(OH)_2D_3$ levels were determined by a competitive binding assay (19) optimized for mouse serum. Briefly, 0.2 ml of mouse serum was extracted with ethyl acetate. After removal of the ethyl acetate, the residue was resuspended in 4% 2-propanol in hexane. The extract was applied to a Sep-Pak Plus silica cartridge (Waters), and was washed with 5% 2-propanol in hexane to remove the 25-hydroxyvitamin D_3 and 24,25-dihydroxyvitamin D_3. The $1,25(OH)_2D_3$ was eluted with 8% 2-propanol in hexane, dried, and resuspended in ethanol. This sample was then used in a competitive binding assay performed with a fixed amount of $1,25(OH)_2[26,27\text{-}^3H]D_3$ and recombinant rat VDR. Serum samples spiked with $1,25(OH)_2[26,27\text{-}^3H]D_3$ were processed and used to determine the percentage recovery.

Kidney Homogenate Preparation. Kidney whole-cell extract was prepared by a modified method of Pierce *et al.* (20) and Sandgren and DeLuca (16). All steps were performed on ice or at 4°C. Kidneys were minced with a razor blade and washed twice with Tris·HCl ethylenediamine-tetraacetic acid dithiothreitol (TED)Na_{150} containing a panel of protease inhibitors. The buffer was decanted and replaced with one volume (vol/vol) of TED plus inhibitors. After homogenization with a Tissue Tearor (Biospec Products, Bartlesville, OK), one volume of $TEDK_{600}Mg_{20}$ was added and homogenization was repeated. Samples were centrifuged at $20,000 \times g$ for 1 h. Supernatant was divided into aliquots, frozen under liquid nitrogen, and stored at −80°C until analysis. The buffers contained the following: 50 mM Tris·HCL, pH 7.4/1.5 mM EDTA/5 mM DTT; 150 mM NaCl or 600 mM KCl/20 mM $MgCl_2$ was added where appropriate. The panel of protease inhibitors (Sigma) consisted of 150 μM aprotinin, 130 μM bestatin, 10 μM leupeptin, 1 μM pepstatin A, and 1 mM phenylmethylsulfonyl fluoride.

Analysis of Kidney Homogenate. VDR content was determined by an ELISA developed in this laboratory (21). The protein concentration of the homogenates was determined by the method of Bradford (22), using BSA as a standard.

RNA Isolation. Total RNA was isolated from mouse kidney with Tri reagent (Molecular Research Center, Cincinnati) according to the manufacturer's protocol.

Quantitative RT-PCR. RNA was DNase-treated (RQ-1 RNase-Free Dnase, Promega) and then reverse transcribed by using the SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). Real-time PCR was performed in a LightCycler (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's recommendations. SYBR green dye was used for quantification of dsDNA after every cycle. The sequence of primers designed for the quantification of β-actin, VDR, 25-hydroxyvitamin D_3-1α-hydroxylase (1α-hydroxylase), and 25-hydroxyvitamin D_3-24-hydroxylase (24-hydroxylase) follows: β-actin (F/R; 5′-3′) TGGAATCCTGTGGCATCCATGAAAC/TAAAACGCAGCTCAGTAACAGTCCG; VDR CTCCTCGATGCCCACCACAAGACCTACG/GTGGGGCAGCATGGAGAGCGGAGACAG; 1α-hydroxylase CCGCGGGCTATGCTGGAAC/CTCTGGGCAAAGGCAAACATCTGA; and 24-hydroxylase TGGGAAGATGATGGTGACCC/ACTGTTCCTTTGGGTAGCGT. All amplicons were 200–500 bp and were sequenced to confirm specificity of amplification. Throughout real-time PCR analysis, product identities were confirmed by melting curve analysis. The quantification of each gene was relative to a standard curve generated from a serially diluted sample. The relative amount of each experimental gene was then normalized to the abundance of the housekeeping gene β-actin. These values were standardized such that a maximum value of 1.0 was assigned to the group with the highest gene expression.

Table 1. Dietary groups, serum calcium, and serum $1,25(OH)_2D_3$ levels

Diet		Serum biochemistry	
Calcium, %	$1,25(OH)_2D_3$, ng/day	Calcium, mg/dl	$1,25(OH)_2D_3$, pg/ml
Vitamin D-deficient group			
0.02	0	5.5 ± 0.3	ND
	50	6.0 ± 0.2	169 ± 78
0.25	0	6.0 ± 0.1	ND
	50	12.6 ± 0.4*	68 ± 10
0.47	0	7.5 ± 0.3	ND
	50	11.2 ± 0.3*	151 ± 9
1.20	0	9.5 ± 0.2	ND
	50	15.8 ± 1.1*	72 ± 32
Vitamin D-replete group			
0.02	0	8.3 ± 0.1†	246 ± 39
	50	8.6 ± 0.1†	419 ± 56
0.25	0	8.6 ± 0.2	195 ± 40
	50	11.3 ± 0.2*	72 ± 11
0.47	0	8.9 ± 0.4	159 ± 16
	50	10.9 ± 0.1*	121 ± 24
1.20	0	9.2 ± 0.3	46 ± 8
	50	12.0 ± 0.2*	124 ± 22

Nonobese diabetic mice were fed a purified diet that contained 0.02%, 0.25%, 0.47%, or 1.20% calcium with or without 50 ng of $1,25(OH)_2D_3$ per mouse per day. Study length was 3 weeks for vitamin D-deficient, calcium-restricted mice and 200 days for all other groups. Serum calcium was determined weekly by atomic absorption spectrometry ($n = 4$). At death, serum was collected and pooled, and the $1,25(OH)_2D_3$ values were determined by a competitive binding assay ($n = 2$–4). All values are reported as the mean ± SEM. ND, not detectable. *, significance at $P < 0.001$ vs. 0 ng groups. †, significance at $P < 0.001$ vs. vitamin D-deficient, 0.02% calcium groups.

Statistical Analysis. The two-tailed Student's t test was used to quantify statistical differences between experimental groups. Results are expressed as mean ± SE.

Results

Serum Analysis. To study the effects of $1,25(OH)_2D_3$ and calcium on renal VDR expression, vitamin D-deficient and vitamin D-replete mice were placed pairwise on diets containing four levels of calcium with or without 50 ng of $1,25(OH)_2D_3$ per day. As indicated in Table 1, the vitamin D-deficient mice on the 0.02% calcium diet had the lowest serum calcium. Dietary supplementation of 50 ng of $1,25(OH)_2D_3$ per day was insufficient to raise serum calcium in these mice, whereas 50 ng of $1,25(OH)_2D_3$ caused a significant increase in serum calcium in all mice that had dietary calcium of at least 0.25%. This finding suggests that a daily dietary intake of 50 ng of $1,25(OH)_2D_3$ is sufficient for vitamin D-induced intestinal calcium uptake, but is insufficient for $1,25(OH)_2D_3$-induced mobilization of calcium from bone. Similar observations were made with the vitamin D-replete mice, although the vitamin D-replete mice on the 0.02% calcium diet had significantly higher serum calcium values than did the calcium-restricted, vitamin D-deficient mice. Because the only source of calcium appearing in the blood of these mice is bone (23), this result demonstrates that vitamin D-replete mice on a calcium-restricted diet can produce sufficient endogenous $1,25(OH)_2D_3$ to mobilize calcium from bone. The serum $1,25(OH)_2D_3$ values suggest that a sufficient amount of $1,25(OH)_2D_3$ to mobilize calcium from bone is ≈250 pg/ml,

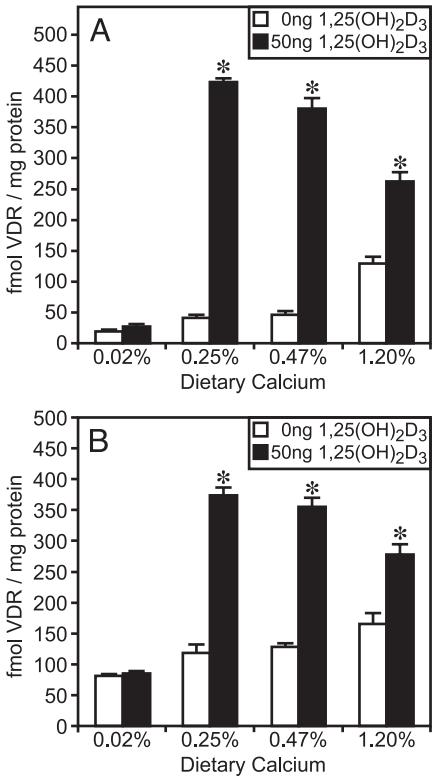

Fig. 1. Regulation of renal VDR by calcium and $1,25(OH)_2D_3$. Mice were fed a purified diet that contained 0.02%, 0.25%, 0.47%, or 1.20% calcium with or without 50 ng of $1,25(OH)_2D_3$ per mouse per day. One kidney per mouse was harvested into isotonic buffer, whole-cell extract was prepared, and VDR content was determined by ELISA and standardized to total protein, as described in *Materials and Methods*. (*A*) Vitamin D-deficient mice, housed under incandescent lighting without dietary intake of vitamin D_3. (*B*) Vitamin D-replete mice, housed under fluorescent lighting and receiving dietary supplementation of vitamin D_3. Each group contained four to six mice. The values represent the mean ± SEM. *, significance at $P \leq 0.01$.

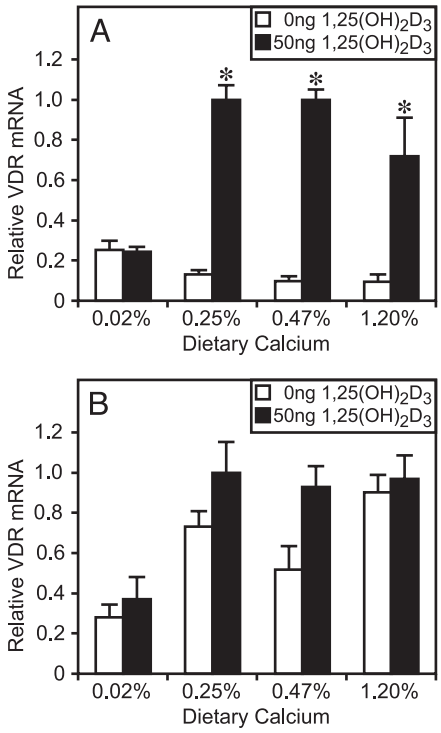

Fig. 2. Regulation of renal VDR mRNA by calcium and $1,25(OH)_2D_3$. Mice were fed a purified diet that contained 0.02%, 0.25%, 0.47%, or 1.20% calcium with or without 50 ng of $1,25(OH)_2D_3$ per mouse per day. One kidney per mouse was harvested into Tri reagent and total RNA was prepared. After reverse transcription, VDR mRNA was quantified by means of real-time PCR and standardized to β-actin mRNA. Values are expressed as relative mRNA with a maximal value of one. (*A*) Vitamin D-deficient mice, housed under incandescent lighting without dietary intake of vitamin D_3. (*B*) Vitamin D-replete mice, housed under fluorescent lighting and receiving dietary supplementation of vitamin D_3. Each group contained four mice. The values represent the mean ± SEM. *, significance at $P \leq 0.01$.

which is well above the amount detected in vitamin D-deficient mice supplemented daily with 50 ng of $1,25(OH)_2D_3$.

Dietary $1,25(OH)_2D_3$ Increases Renal VDR When Dietary Calcium Is Present. In both vitamin D-deficient and vitamin D-replete mice, daily dietary intake of 50 ng of $1,25(OH)_2D_3$ was sufficient to significantly increase renal VDR when at least 0.25% calcium was present in the diet (Fig. 1). These recorded increases ranged from 1.7-fold for the vitamin D-replete, 1.20% dietary calcium mice to 10.3-fold for the vitamin D-deficient, 0.25% calcium group. Daily consumption of 50 ng of $1,25(OH)_2D_3$ did not increase renal VDR in mice fed a calcium-restricted diet. In fact, the mice on the 0.02% calcium diets had reduced levels of VDR expression. Vitamin D-deficient mice on a calcium-restricted diet had renal VDR values of 19–27 fmol/mg protein, whereas vitamin D-replete mice on the calcium-restricted diet had renal VDR values of 81–85 fmol/mg protein. These studies demonstrate that both $1,25(OH)_2D_3$ and calcium regulate mouse renal VDR expression.

$1,25(OH)_2D_3$ and Calcium Are Required to Activate VDR Transcription. To further our understanding of the regulation of renal VDR by $1,25(OH)_2D_3$ and calcium, we performed real-time PCR experiments to quantify relative VDR mRNA between dietary groups. The VDR mRNA in vitamin D-deficient mice was expressed at a low basal level, regardless of dietary calcium (Fig. 2). Vitamin D-deficient mice supplemented with 50 ng of $1,25(OH)_2D_3$ showed a dramatic 8- to 10-fold increase in renal VDR transcript levels when at least 0.25% calcium was present in the diet. $1,25(OH)_2D_3$ supplementation did not increase renal VDR mRNA in mice fed the calcium-restricted diet. Combined, these studies demonstrate that both $1,25(OH)_2D_3$ and calcium are required for expression of renal VDR mRNA above basal levels.

In vitamin D-replete mice, the VDR mRNA levels were lowest in the 0.02% calcium group, confirming the requirement of calcium for renal VDR mRNA expression. In the calcium-restricted mice, supplementation with $1,25(OH)_2D_3$ did not significantly increase the abundance of VDR transcript, likely because of the lack of calcium. Mice fed calcium-fortified diets expressed consistently high levels of relative VDR transcript because of their endogenous production of $1,25(OH)_2D_3$ and consumption of calcium. This study further suggested that $1,25(OH)_2D_3$ and calcium are required to activate VDR transcription and increase the relative VDR mRNA.

The 1α-Hydroxylase and 24-Hydroxylase Are Reciprocally Regulated. To further characterize the metabolic status of the mice in this study, the transcript levels of the two primary vitamin D metabolism enzymes were quantified. The 1α-hydroxylase is responsible for the conversion of 25-hydroxyvitamin D_3 to $1,25(OH)_2D_3$. This enzyme is primarily expressed in the kidney, and is stimulated by parathyroid hormone (PTH), and suppressed by $1,25(OH)_2D_3$ (24, 25). As was expected, the 1α-hydroxylase transcript levels were highest in the vitamin D-deficient mice, and in the mice fed the calcium-restricted diets

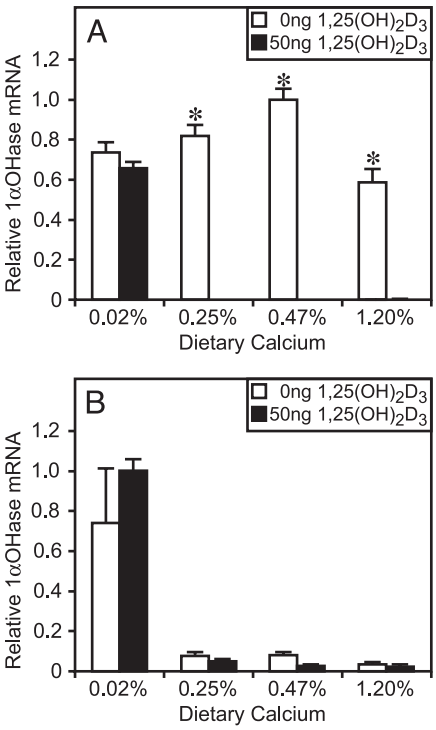

Fig. 3. Regulation of renal 1α-hydroxylase mRNA by calcium and $1,25(OH)_2D_3$. Mice were fed a purified diet that contained 0.02%, 0.25%, 0.47%, or 1.20% calcium with or without 50 ng of $1,25(OH)_2D_3$ per mouse per day. One kidney per mouse was harvested into Tri reagent, and total RNA was prepared. After reverse transcription, 1α-hydroxylase mRNA was quantified by means of real-time PCR and standardized to β-actin mRNA. Values are expressed as relative mRNA with a maximal value of one. (*A*) Vitamin D-deficient mice, housed under incandescent lighting without dietary intake of vitamin D_3. (*B*) Vitamin D-replete mice, housed under fluorescent lighting and receiving dietary supplementation of vitamin D_3. Each group contained four mice. The values represent the mean ± SEM. *, significance at $P \leq 0.01$.

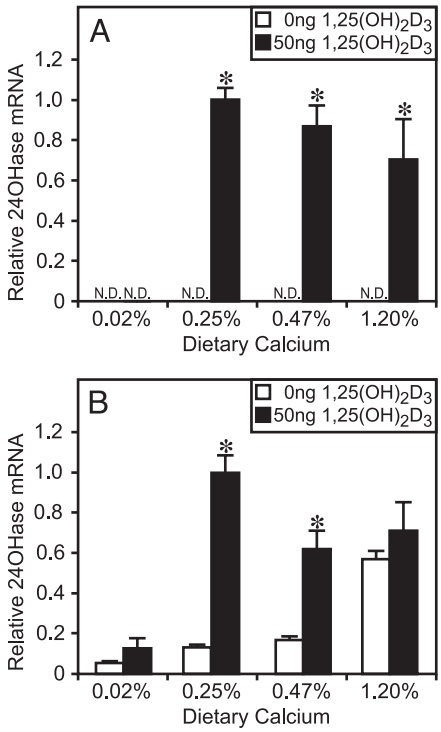

Fig. 4. Regulation of renal 24-hydroxylase mRNA by calcium and $1,25(OH)_2D_3$. Mice were fed a purified diet that contained 0.02%, 0.25%, 0.47%, or 1.20% calcium with or without 50 ng of $1,25(OH)_2D_3$ per mouse per day. One kidney per mouse was harvested into Tri reagent, and total RNA was prepared. After reverse transcription, 24-hydroxylase mRNA was quantified by means of real-time PCR and standardized to β-actin mRNA. Values are expressed as relative mRNA with a maximal value of one. (*A*) Vitamin D-deficient mice, housed under incandescent lighting without dietary intake of vitamin D_3. (*B*) Vitamin D-replete mice, housed under fluorescent lighting and receiving dietary supplementation of vitamin D_3. Each group contained four mice. The values represent the mean ± SEM. ND, not detectable. *, significance at $P \leq 0.01$.

(Fig. 3). These mice had the lowest serum calcium values, and likely expressed high amounts of PTH, because PTH expression is inversely related to serum calcium (26). In the vitamin D-deficient study, 50 ng of $1,25(OH)_2D_3$ nearly completely abolished 1α-hydroxylase expression in mice consuming dietary calcium. It is difficult to separate the direct effects of $1,25(OH)_2D_3$ on repression of the 1α-hydroxylase gene from the secondary effects of increased serum calcium and decreased PTH. In the vitamin D-replete study, 50 ng of $1,25(OH)_2D_3$ had minimal impact on relative 1α-hydroxylase mRNA in mice consuming dietary calcium, because the 1α-hydroxylase expression was consistently low due to endogenous production of $1,25(OH)_2D_3$.

The 24-hydroxylase is the major catabolic enzyme responsible for the degradation of $1,25(OH)_2D_3$. The 24-hydroxylase promoter contains two vitamin D-response elements and has been well characterized as a target gene for activation by $1,25(OH)_2D_3$ (27, 28). 24-Hydroxylase mRNA was undetectable in vitamin D-deficient mice, further confirming the vitamin D-deficient status of these mice (Fig. 4). Vitamin D-deficient mice receiving 50 ng of $1,25(OH)_2D_3$ produced significant amounts of 24-hydroxylase mRNA only if they were consuming dietary calcium. This finding was almost certainly due to the fact that up-regulation of the 24-hydroxylase by $1,25(OH)_2D_3$ is receptor dependent, and, as was shown in Fig. 1, the VDR expression in the vitamin D-deficient, calcium-restricted mice was very low. In the calcium-restricted, vitamin D-replete mice, 24-hydroxylase transcript was low yet detectable because of the very high levels of $1,25(OH)_2D_3$ (Table 1) and low but sufficient expression of VDR. When at least 0.25% calcium was present in the diet, supplementation with $1,25(OH)_2D_3$ resulted in a significant increase in the 24-hydroxylase mRNA. This increase was not significant for the vitamin D-replete 1.20% calcium group, because these mice had elevated serum calcium values and, thus, had relatively high 24-hydroxylase mRNA levels without supplementation of $1,25(OH)_2D_3$. The reciprocal regulation of the renal 1α-hydroxylase and 24-hydroxylase observed in this study further demonstrates the influence of $1,25(OH)_2D_3$ and calcium on the regulation of vitamin D hormone levels.

Discussion

We have conducted a comprehensive, long-term study examining the effects of $1,25(OH)_2D_3$ and dietary calcium on mouse renal VDR expression. In this study we have shown that 50 ng of $1,25(OH)_2D_3$ can cause a 10-fold increase in the expression of renal VDR in a vitamin D-deficient mouse. Previous studies (12, 14, 17) in vitamin D-deficient rats have shown a 2- to 4-fold increase in renal VDR content in response to $1,25(OH)_2D_3$. The more dramatic increase observed in this study may be due to species differences, variances in dosage and administration (dietary versus injection or osmotic pump), or the significantly greater length of this 200-day study. In vitamin D-deficient rats, Uhland-Smith and DeLuca (17) did observe a greater increase in VDR expression after 8 weeks of $1,25(OH)_2D_3$ treatment

(4-fold increase versus controls) than after 4 weeks (2-fold increase versus controls) of $1,25(OH)_2D_3$ treatment.

In the absence of dietary calcium, VDR levels are diminished to <30 fmol/mg protein in the vitamin D-deficient mouse and $1,25(OH)_2D_3$ supplementation did not increase expression. In vitamin D-deficient rat studies, calcium restriction caused renal VDR content to decrease to 50–100 fmol/mg protein, and $1,25(OH)_2D_3$ could not increase VDR expression (16, 17).

To investigate the extent to which $1,25(OH)_2D_3$ and calcium regulate renal VDR at the transcriptional level, VDR mRNA was quantified by real-time PCR. Because the VDR mRNA is of relative low abundance and comigrates with ribosomal RNA during gel electrophoresis, quantification of the transcript by Northern analysis can be difficult. Real-time PCR is sensitive, as well as quantitative, for a large number of samples. Analysis demonstrated that the VDR mRNA is expressed at a low basal level in the absence of either $1,25(OH)_2D_3$ or calcium. The addition of 50 ng of $1,25(OH)_2D_3$ to the diet of a vitamin D-deficient mouse resulted in an 8- to 10-fold increase in the amount of VDR mRNA when calcium was present in the diet. This increase was completely absent in mice fed a calcium-restricted diet, clearly demonstrating that both $1,25(OH)_2D_3$ and calcium are required for VDR mRNA expression above a basal level in mouse kidney. Whereas previous reports have concluded that $1,25(OH)_2D_3$ can increase VDR mRNA in the parathyroid gland of vitamin D-deficient chickens and rats, little or no change has been observed in kidney VDR mRNA (10, 13, 18). The studies that examined changes in renal VDR mRNA in response to $1,25(OH)_2D_3$ administration ranged from a single i.p. injection (10) to daily injections for one week (13). Because the half-life of $1,25(OH)_2D_3$ is <6 h in the mouse (29), it is possible that daily injections of $1,25(OH)_2D_3$ did not alter the transcript level, or caused a temporal increase that was not detected by the Northern analysis. In the longer term studies that explored the regulation of rat renal VDR by $1,25(OH)_2D_3$ and calcium, VDR mRNA values were not reported (14, 17).

The kidney is a vitamin D target tissue critical in the reabsorption of urinary calcium. The increase in renal VDR caused by $1,25(OH)_2D_3$ is most likely a result of increased transcription and ligand-induced stabilization, and could serve as a means of signal amplification. The kidney is also an important tissue for the regulation of the metabolism of $1,25(OH)_2D_3$, because both the 1α-hydroxylase and 24-hydroxylase are expressed in the renal proximal tubular cells. Down-regulation of renal VDR in response to hypocalcemia may be a protective measure to block $1,25(OH)_2D_3$-mediated suppression of the 1α-hydroxylase and induction of the 24-hydroxylase, resulting in a net increase in serum $1,25(OH)_2D_3$ levels (15).

The molecular mechanism by which $1,25(OH)_2D_3$ and calcium regulate renal VDR expression remains unclear. These experiments demonstrate that $1,25(OH)_2D_3$ and calcium are both required to increase renal VDR mRNA levels. Sequence analysis of the mouse and human VDR promoters does not reveal any vitamin D response elements, and no response to $1,25(OH)_2D_3$ has been detected during reporter-gene analysis, suggesting that $1,25(OH)_2D_3$ does not directly activate expression of its receptor (30–32). Further experiments must be performed to discern the extent of transcriptional and posttranscriptional regulation, identify regulatory pathways and signaling molecules, and elucidate the mechanism behind the tissue specificity of this regulation.

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