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Serum calcium and vitamin D regulate 1,25-dihydroxyvitamin D_3 receptor concentration in rat kidney *in vivo*

(phosphate/steroid receptor)

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ABSTRACT **The effects of vitamin D status, serum calcium, and serum phosphorus levels on 1,25-dihydroxyvitamin D receptor levels in kidney were investigated. Weanling rats were fed for 4 weeks on a diet with various levels of calcium and phosphorus with or without vitamin D. The 1,25-dihydroxyvitamin D_3 receptor concentration in kidney was determined by an immunoradiometric assay. In the absence of vitamin D, total receptor concentration is increased 2-fold by an increase in serum calcium concentration. At normal serum calcium levels, the administration of vitamin D resulted in a 5-fold increase in receptor concentration. In hypocalcemic animals, however, vitamin D did not change receptor levels. Serum phosphorus levels could not be linked to any changes in 1,25-dihydroxyvitamin D_3 receptor concentration. This study demonstrates that serum calcium levels and vitamin D regulate 1,25-dihydroxyvitamin D_3 receptor concentration *in vivo* in kidney. On the other hand, vitamin D is unable to exert control of receptor levels in kidney under hypocalcemic conditions.**

1,25-Dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] is the main regulator of plasma calcium and phosphorus levels (1). Vitamin D_3 undergoes a two-step hydroxylation, first in the liver and then in the kidney, to form the active metabolite 1,25-$(OH)_2D_3$. The hormone acts through an intracellular receptor protein to modulate specific gene transcription in target tissues (2).

One key to a better understanding of the physiological responses to vitamin D is to study regulation of the 1,25-$(OH)_2D_3$ receptor (VDR). The importance of receptor regulation is emphasized by the observations that biological response to 1,25-$(OH)_2D_3$ is a direct function of receptor number (3) and occupancy (4, 5). Previous work in cell culture systems has shown that 1,25-$(OH)_2D_3$ up-regulates its own receptor (6). Regulation of the receptor has also been studied *in vivo* in the rat (7, 8). Measured by ligand binding, 1,25-$(OH)_2D_3$ was found to have no effect on intestinal receptor levels after 24 hr and caused only a marginal increase after 5 days of treatment. The VDR in kidney, however, was significantly up-regulated at both time points.

By using an immunoradiometric assay (IRMA) for total vitamin D_3 receptor protein (9), we have shown a 2-fold increase in receptor protein levels in rat intestine *in vivo* 12 hr after intravenous administration of 1,25-$(OH)_2D_3$, although no change was detected by ligand binding (8).

In this study we examined the effects of dietary vitamin D, serum calcium, and serum phosphorus on VDR concentration in rat kidney *in vivo* using an IRMA. We have found that calcium itself up-regulates the VDR concentration and that calcium plus vitamin D causes an impressive increase in receptor levels.

MATERIALS AND METHODS

Animals. Male weanling rats were obtained from Sprague Dawley (Holtzmann strain, Madison, WI) and kept on different diets for 4 weeks. Vitamin D-deficient rats were supplemented with vitamins A, E, and K; vitamin D-sufficient rats received the same diet but with the addition of 75 international units of vitamin D_2 three times weekly. Animals were considered vitamin D-deficient when serum calcium fell below 6 mg/dl in control rats fed a diet containing 0.3% phosphorus, 0.47% calcium, and no vitamin D.

Buffers. The following buffers were used: phosphate-buffered saline (PBS), 1.5 mM KH_2PO_4/8.1 mM Na_2HPO_4, pH 8.0/137 mM NaCl/2.7 mM KCl; PBS/Triton, 0.5% Triton X-100/PBS; TE, 50 mM Tris·HCl, pH 7.4/1.5 mM EDTA; TED, 5 mM dithiothreitol/TE; TEDK300, 300 mM KCl/TED; TEDK300/bovine serum albumin, 0.5% bovine serum albumin/0.02% NaN_3/TEDK300; TEDNa150, 150 mM NaCl/5 mM dithiothreitol/TED; homogenization buffer, 5 mM diisopropyl fluorophosphate/TEDK300.

Sample Collection. Animals were anesthesized with ether and tail-bled for serum calcium and serum phosphorus determinations. Kidneys were removed and put in ice-cold TEDNa150.

Sample Preparation. Kidneys were decapsulated and minced on ice with a razor blade, washed three times with TEDNa150, and weighed. Tissue was then homogenized on ice in 2 vol (wt/vol) homogenization buffer using a glass–Teflon homogenizer. Cytosol was obtained by ultracentrifugation for 1 hr at 170,000 × *g* and subsequently divided, frozen in liquid nitrogen, and stored at −70°C until use.

IRMA. IRMA was performed as described (9). Briefly, a sample of receptor or standard receptor was incubated overnight at 4°C with radioiodinated anti-receptor antibody and with a second anti-receptor antibody, directed to a different epitope, that is biotinylated. The antibody–receptor complexes were then precipitated with avidin-Sepharose and washed three times with PBS/Triton. Radioactivity was measured in a Packard Multi-Prias auto γ counter (Packard Instrument). The amount of precipitated radioiodinated antibody is directly related to the amount of receptor in the sample or standard.

Protein was measured with the Bio-Rad microassay (Bio-Rad) using bovine serum albumin (Sigma) as a standard. Serum calcium was determined in the presence of 0.1% $LaCl_3$ by using a Perkin–Elmer model 403 atomic absorption spectrometer. Serum phosphorus was determined by the method of Itaya *et al.* (10). Statistical analysis was performed using Student's *t* test.

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Abbreviations: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; VDR, 1,25-$(OH)_2D_3$ receptor.

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RESULTS

To examine if calcium plays a role in VDR regulation, we studied hypocalcemic and normocalcemic rats in the presence or absence of dietary vitamin D (Table 1). In this study, rats were divided into two major groups: Group A was fed a purified diet with 0.3% phosphorus and a calcium concentration of 3.0%, 0.47%, or 0.04% plus or minus vitamin D_2. This resulted in four subgroups, respectively: +D normocalcemic, −D normocalcemic, +D hypocalcemic, and −D hypocalcemic. Group B was fed a purified diet with 1.2% calcium and 1.4%, 0.3%, or 0.1% phosphorus, resulting in the subgroups: +D normophosphatemic, −D normophosphatemic, and −D hypophosphatemic. In the vitamin D-deficient rat, we found that a change in serum calcium from 4.9 to 10.6 mg/dl increased VDR levels approximately 2-fold (66 to 149 fmol/mg of protein, $P < 0.001$) (Fig. 1). A similar increase in serum calcium in rats given vitamin D caused a 5-fold up-regulation of the receptor (52 to 286 fmol of receptor per mg of protein, $P < 0.001$).

By comparing hypocalcemic rats in the presence or absence of dietary vitamin D, we found that vitamin D had no effect on VDR levels in the kidney in these rats. The vitamin D-deficient and vitamin D-sufficient, hypocalcemic animals expressed 66 and 52 fmol of receptor protein, respectively. When normocalcemic D-depleted rats were compared with normocalcemic D-repleted rats, an up-regulation of VDR from 149 (−D) to 275 (+D) fmol was observed ($P < 0.001$) (Fig. 1).

We also investigated the effect of low and normal serum phosphorus levels on VDR concentration in the normocalcemic rat (Table 1). In the D-deficient animal, VDR levels were unchanged by an increase in serum phosphorus concentration from 2.3 to 7.2 mg/dl (Fig. 2). The hypophosphatemic group expressed 188 fmol and the normophosphatemic group 179 fmol of receptor protein, respectively. Vitamin D caused a marked increase in receptor number from 179 to 410 fmol/mg of protein in this group ($P < 0.001$) (Fig. 2).

DISCUSSION

VDRs are low-abundance intracellular proteins that belong to the "superfamily" of steroid hormone receptors (11). Many factors have been reported to modulate VDR expression including glucocorticoids (12), age (13), parathyroid hormone (14), developmental stage (15), and the cognate ligand 1,25-$(OH)_2D_3$ (6, 16). Other steroid hormones, such as glucocorticoids and estrogens, have been reported to down-regulate their respective receptors (17, 18). For vitamin D, homologous up-regulation of the receptor protein has been shown both *in vitro* (16) and *in vivo* (8), and there is evidence that the regulation occurs, at least in part, at the level of transcription (19, 20).

Table 1. Serum calcium and serum phosphorus of animals studied

Experimental group	Calcium, mg/dl	Phosphate, mg/dl
Group A		
+D normocalcemic	11.5 ± 0.3	7.2 ± 0.6
−D normocalcemic	10.6 ± 0.2	0.5 ± 0.1
+D hypocalcemic	5.1 ± 0.2	8.1 ± 0.2
−D hypocalcemic	4.9 ± 0.2	7.0 ± 0.5
Group B		
+D normophosphatemic	10.8 ± 0.2	7.0 ± 0.2
−D normophosphatemic	10.5 ± 0.2	7.2 ± 0.4
−D hypophosphatemic	11.2 ± 0.1	2.3 ± 0.4

Each group represents data from at least five animals and values are expressed as the mean ± SEM.

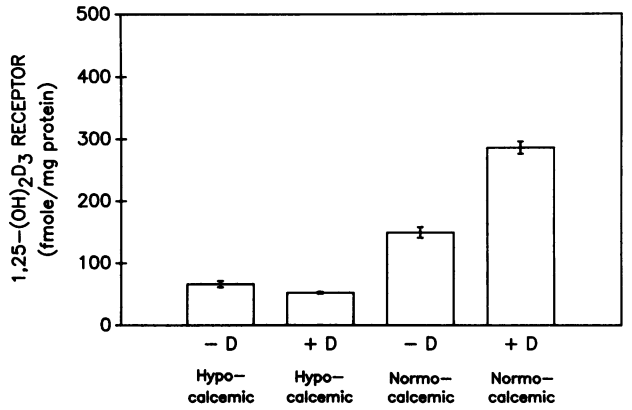

FIG. 1. Effect of serum calcium on VDR protein levels in the presence (+D) or absence (−D) of vitamin D. VDR levels in rat kidney cytosols were measured by an IRMA. Each sample represents data from five or more animals (mean ± SEM).

In the absence of a normal serum calcium, we have demonstrated that dietary vitamin D does not alter VDR levels in rat kidney *in vivo*. Our results also indicate that serum calcium up-regulates VDR both in the presence and absence of vitamin D. Thus, calcium and not 1,25-$(OH)_2D_3$ may also mediate the 1,25-$(OH)_2D_3$-induced up-regulation of VDR. In contrast to calcium, we found that serum phosphorus levels did not regulate receptor levels.

Costa *et al.* (16) demonstrated an up-regulation of VDRs in kidney in D-deficient rats after treatment with 1,25-$(OH)_2D_3$. However, the possible role of serum calcium in this event was not investigated. Data from previous experiments in our laboratory (8) have shown an increase in VDR protein and mRNA levels in rat intestine *in vivo* after a single injection of 1,25-$(OH)_2D_3$. However, we did not exclude the possibility that this response is mediated by calcium. It is clear that an increase in calcium transport in intestine after hormone treatment precedes the up-regulation of VDRs by several hours (21, 22), thus calcium could well be the mediator in this up-regulation.

The mechanisms underlying the calcium-induced increase in VDR level are unknown. One possible mechanism could be through the parathyroid hormone (PTH) since it has been reported that PTH up-regulates the VDR (14). Because normal or high serum calcium suppresses PTH secretion, this

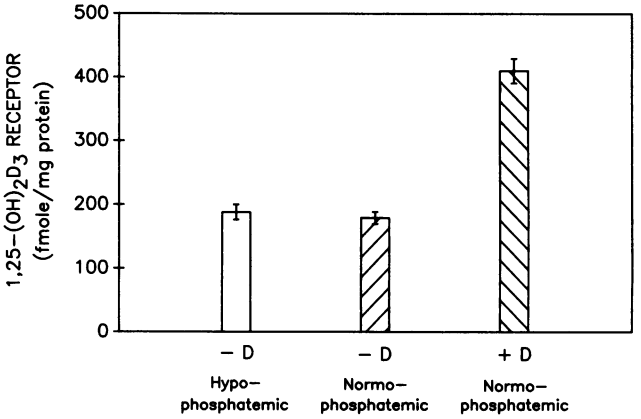

FIG. 2. Effect of serum phosphorus on VDR protein levels in the presence (+D) or absence (−D) of vitamin D. VDR content in rat kidney cytosols was determined by IRMA. A minimum of five animals was used for each group and all values are expressed as mean ± SEM.

hypothesis is rather unlikely (2). We have also noted in experiments with parathyroidectomized rats that PTH did not seem to regulate VDR expression in rat kidney *in vivo* (data not shown). Calcium may also act as a transcriptional factor either through a direct effect of free calcium ions on DNA or through binding to an intracellular protein, forming a complex that enhances VDR gene transcription.

It is curious that kidney appears more sensitive to VDR regulation than duodenum. Since receptor-mediated regulation of vitamin D metabolism is a prominent feature of the kidney, receptor regulation may be related to the need for sensitive inactivation of 1,25-$(OH)_2D_3$ and 25-hydroxyvitamin D_3. Under hypocalcemic conditions, high levels of 1,25-$(OH)_2D_3$ are needed, diminishing the need for inactivation and metabolism. We believe that the magnitude of change in VDR levels observed in this study is, indeed, physiologically relevant. A parallel can be drawn with the glucocorticoid receptor where a modest reduction in glucocorticoid receptor protein levels is enough to lower cellular glucocorticoid responsiveness significantly (23).

It is clear that VDR regulation is more complex than previously appreciated. Thus, it is not clear what is the primary regulator of VDR and what is the physiologic value of regulating this receptor. These findings, therefore, open an important area of study in calcium metabolism.

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