Selective Biological Response by Target Organs (Intestine, Kidney, and Bone) to 1,25-Dihydroxyvitamin D3 and Two Analogues

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ABSTRACT

The hormonally active form of vitamin D, 1α,25-dihydroxyvitamin D3 [1α,25(OH)2D3], stimulates biological responses related to calcium homeostasis, cell differentiation, and immunomodulation in many target cells, including leukemic cells. Most of these responses are dependent upon 1α,25(OH)2D3 interaction with a nuclear receptor protein. Structural analogs of 1α,25(OH)2D3 might allow for separation of biological function, avoiding adverse calcemic effects. This report quantitates intestinal calcium absorption, bone calcium resorption, induction of intestinal and renal calcium-binding protein (CaBP), and occupancy of the intestinal and renal nuclear 1α,25(OH)2D3 receptor in vitamin D-deficient chicks after a single dose of 1α,25(OH)2D3. 1α,25-dihydroxyvitamin-16-ene-23-yne-D3 (analogue V), or 22-[m-(dimethylhydroxymethyl)phenyl]-23,24,25,26,27-pentanoro-1α-hydroxy-vitamin D3 (analogue EV).

The interaction of these compounds with chick intestinal nuclear 1α,25-(OH)2D3 receptor and chick plasma D-binding protein was determined in vitro; analogues V and EV bound 68% and 62% [1α,25(OH)2D3 receptor] and 8% and 13% (vitamin D-binding protein), respectively, as well as 1α,25(OH)2D3 (100%). 1α,25(OH)2D3 doses (0.075-1.2 nmol) generated responses in intestinal calcium absorption, bone calcium resorption, intestinal CaBP, and renal CaBP. When analogue V (1.2-300 nmol) was administered, increases in bone calcium resorption and renal CaBP were noted. However, a significant response in intestinal calcium absorption and intestinal CaBP appeared only after a 300-nmol dose. Unoccupied nuclear 1α,25(OH)2D3 receptor in the intestine and kidney was determined in vivo after doses of 1α,25(OH)2D3 analogue V, or analogue EV. Doses (0.25-6.0 nmol) of 1α,25(OH)2D3 and analogue EV reduced unoccupied receptor to 24% and 59% (intestine) and to 13% and 41% (kidney), respectively. Analogue V (6.0-600 nmol) decreased unoccupied receptor in the kidney. In the intestine analogue V (300-600 nmol) reduced unoccupied receptor only to 75%. These results confirm that some vitamin D analogues can generate selective biological responses and different levels of target organ receptor occupancy.

INTRODUCTION

1α,25(OH)2D3, the hormonally active form of vitamin D3 (1, 2), is capable of generating a wide spectrum of biological responses. These include stimulation of ICA, BCM (3), repression of secretion of parathyroid hormone (4), stimulation of transcalcitina (defined as the very rapid, within 2-10 min, stimulation of intestinal calcium transport) (5, 6), opening of calcium channels in osteoblast cells (7), promotion of differentiation of the promyelocytic cell line HL-60 into mature monocytes and macrophages (8), and possibly osteoclasts (9), and stimulation of normal insulin secretion (10). Many of these biological responses are believed to occur as a consequence of the regulation of gene transcription by the classical nuclear VDR (11), although some responses are known to occur via nongenomic mechanisms (6, 12).

Given the diversity of biological responses generated by 1α,25-(OH)2D3, the important question arises of whether the signal transduction mechanisms for 1α,25(OH)2D3 have identical or different ligand structural requirements. Currently, it is generally assumed that there is only one form of the classical nuclear VDR (13), which might imply that all target tissues would display identical vitamin D ligand specificities. Thus, the intestine, bone, and kidney all might be predicted to display similar dose-response relationships for VDR-mediated biological responses. Intriguingly, there are several reports of differential biological responses to several analogues of 1α,25-(OH)2D3. Thus, 1α,25(OH)2D3 and 1α,25(OH)2D3-22-oxa-vitamin D3 (4, 14). 1α,25(OH)2-22,24-cyclopolyvitamin D3 (15), analogue V (16, 17), and 1α,25(OH)2-vitamin D3-24-homo analogues (18, 19) display a dissociation of calcitropic actions from their abilities to promote cell differentiation. Analogue EV might also be useful in cell differentiation or suppress parathyroid hormone secretion, without hypercalcemic action, could be useful clinically.

To date, while several studies have reported the biological activity of 1α,25(OH)2D3 analogues in several target organs of the same animal, no direct comparison of the extent of vitamin D receptor occupancy with the extent of biological response has been achieved. In this report we examine the spectrum of biological responses (ICA, BCM, and induction of calbindin-D28K in intestine and kidney) in vitamin D-deficient chicks after administration of 1α,25(OH)2D3 and the two analogues analogue V and analogue EV and correlate these responses with VDR occupancy. Our results indicate that some analogues of 1α,25(OH)2D3 generate differential biological responses in various target organs in vivo.

MATERIALS AND METHODS

Chemicals. Analogue EV was synthesized as described by Figadère et al. (20). 1α,25(OH)2D3 and analogue V were the gift of Dr. M. R. Uskokovic (Hoffmann-LaRoche, Nutley, NJ). 1α,25(OH)2[23,24(N)-3H]cholecalciferol (specific activity, 97 Ci/mmol) was obtained from Amersham Corp. (Arlington Heights, IL). Other laboratory reagents were of the highest purity available. Animals and Tissue Preparations. White Leghorn cockerels (Lakeview Farms, Lakeview, CA) were obtained on the day of hatch and raised for 3-4 weeks on a standard rachiogen diet (21). All experiments using animals were approved by the University of California, Riverside, Chancellor's Committee on Animal Research.

Subsequent treatments in individual experiments are shown in the table and figure legends. Test compounds were injected i.m. in a mixture of ethanol:1,2-propanediol (1:1, v/v). After decapitation, the small intestine and kidneys were removed and washed twice with ice-cold phosphate-buffered saline. All subsequent steps were performed at 0-4°C. The intestinal mucosa was collected by scraping, kidneys were minced, and the tissues were homogenized (10%, w/v) in a low ionic strength buffer (TED) with a motor-driven glass/Teflon homogenizer. The homogenate was centrifuged at 5000 X g for 10 min. The nuclear pellet was washed twice with vigorous vortexing in the same volume of TED plus 0.5% Triton X-100. The washed chromatin pellet was
resuspended (10% tissue, w/v) in a high ionic strength buffer (TED containing 0.3 mM KCl). The suspension was incubated on ice for 30 min and then centrifuged at 10,000 × g for 10 min. The resulting supernatant fraction (chromatin extract) was used, freshly prepared, for the [3H]-1α,25(OH)2D3 binding assay.

Quantitation of 1α,25(OH)2D3 Receptors. Quantitation of unoccupied VDR was carried out as described previously (22, 23). Aliquots (100 μl, 0.3–0.4 mg protein) of the TED-KCl chromatin extract of VDR from chick intestine or kidney were pipetted into polypropylene tubes containing [3H]-1α,25(OH)2D3 (0.125–2.0 nM), in the absence (total binding) or presence (nonspecific binding) of a 250-fold excess of nonradioactive 1α,25(OH)2D3. The tubes were incubated overnight (16–18 h) at 4°C. At the end of the incubation, 200 μl of hydroxylapatite suspension (50%, v/v, in TED) were added to the tubes to separate bound from free steroid and the incubation was continued for 15 min at 4°C, with vortexing. After addition of 1 ml TED-Triton buffer, the tubes were vortexed and centrifuged at 1000 × g for 5 min; the resulting pellets were washed twice with TED-Triton buffer. The radioactivity of the final pellets was extracted with ethanol; the supernatants were air dried and counted. For each tissue sample a five-point saturation analysis was carried out and the specific 1α,25(OH)2D3 binding was analyzed (24); the values of βmax and receptor-steroid dissociation constant for the VDR were calculated via the line obtained for the analogue is divided by the slope of the line obtained

Apart from the increase in total serum calcium concentration, as determined by

Results

The principal objective of this study was to compare the biokinetics of 1α,25(OH)2D3 receptor occupancy and onset of biological responses after administration of 1α,25(OH)2D3 (analogue C) and its analogues analogue V and analogue EV. These results are considered in relation to the ability of 1α,25(OH)2D3 and its analogues to bind in vitro to the plasma transport DBP and to the intestinal or kidney VDR. The structures of 1α,25(OH)2D3 and the two test analogues are shown in Fig. 1.

Table 1 reports the determination of the RCI for the three test compounds for both the nuclear VDR and the plasma DBP. In these evaluations the RCI for 1α,25(OH)2D3 is, by definition, 100. It is apparent that, for binding to the chick intestinal receptor, both analogue V and analogue EV are quite effective ligands, possessing RCIs of 68 and 62, respectively. With respect to binding to DBP, the test analogues V and EV have RCIs of 8.6 and 13, respectively; thus, the test analogues V and EV would be expected to have high "free" concentrations in vivo and, accordingly, would be more available for target organ uptake than the reference 1α,25(OH)2D3.

Fig. 2 shows the ability of the two test analogues of 1α,25(OH)2D3 to stimulate ICA and BCM under our standard conditions in vitamin D-deficient chicks. In these assays, a negative control (vehicle) and a positive control (3.0 nmol of vitamin D3) were administered to define the minimum and maximum anticipated responses, respectively, of ICA and BCM. Increasing doses of 1α,25(OH)2D3, analogue V, and analogue EV were given 12 h before assay. As anticipated, physiological doses of 1α,25(OH)2D3 (0.06–1.2 nmol) were quite effective in Fig. 1.

1α,25(OH)2D3 Receptor and Vitamin D-binding Protein Steroid Competition Assays. The assay of competitive binding of analogues V and EV to the chick intestinal VDR was performed using the hydroxylapatite batch assay (25). Increasing amounts of nonradioactive 1α,25(OH)2D3 or analogue were added to a constant amount of [3H]-1α,25(OH)2D3, and incubated with chick intestinal cytosol. The RCI for 1α,25(OH)2D3 analogues was calculated by plotting the percentage of maximum [3H]-1α,25(OH)2D3 bound on the ordinate versus [competitor]/[3H]-1α,25(OH)2D3 on the abscissa. The slope of the line obtained for the analogue is divided by the slope of the line obtained for 1α,25(OH)2D3; multiplication of this value by 100 results in the RCI. By definition, the RCI for 1α,25(OH)2D3 is 100.

Enzyme-linked Immunoassay for Calbindin-D28K. Calbindin-D28K (CaBP) content in the intestinal mucosa and kidneys of chicks was determined by the enzyme-linked immunosorbent assay method (28, 27). Rabbit antiserum against highly purified chick intestinal calbindin-D28K was used as a source of the first antibody and anti-rabbit IgG alkaline phosphatase conjugate was used as the second antibody. Intestinal and renal cytosolates were obtained by centrifugation (35,000 × g for 1 h at 4°C) of TED homogenates (10%, w/v) of intestinal mucosa and kidneys. Before CaBP assay, the cytosol was diluted 1:50 in the case of intestinal mucosa or 1:5 in the case of kidneys.

Assays of Intestinal Calcium Absorption and Bone Calcium Mobilization. The determination of ICA and BCM was carried out in vitamin D-deficient chicks according to our previously published procedures (3, 16). Twelve h before assay, the chicks, which had been placed on a diet containing no calcium 48 h before assay, were given i.m. injections of the vitamin metabolite or analogue dissolved in ethanol:1,2-propanediol (1:1, v/v) or of vehicle. At the time of assay, 4.0 mg of 45Ca2+ plus 5 μCi of 45Ca2+ were placed in the duodenum of the birds, which had been lightly anesthetized with ether. After 30 min, the birds were decapitated and the blood was collected. The radioactivity content of 0.2 ml of serum was measured to determine the amount of 45Ca2+ absorbed (which is a measure of ICA). BCM activity was estimated from the increase in total serum calcium concentration, as determined by atomic absorption spectrophotometry (model 503; Perkin-Elmer, Norwalk, CT).

Statistics. Data are expressed as mean ± SE. Statistical significance was determined by Student's t-test, paired or unpaired as appropriate.

RESULTS

The structures of 1α,25(OH)2D3 (analogue C), analogue V, and analogue EV. Fig. 1.
Table 1. Relative competitive index for 1α,25(OH)2D3 and its analogues for chick intestinal receptor and chick plasma vitamin D-binding protein

<table>
<thead>
<tr>
<th>Compound</th>
<th>Analogue code</th>
<th>RCI</th>
<th>Intestinal VDR</th>
<th>DBF (a, b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α,25(OH)2D3</td>
<td>C</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>1α,25(OH)2-16-cene-23-yne-vitamin D3</td>
<td>V</td>
<td>68 ± 3</td>
<td>8.6 ± 1.5</td>
<td></td>
</tr>
<tr>
<td>22-[β-(Dimethylamino)phenyl]-23,24,25,26,27-pentanor-1α-hydroxy-vitamin D3</td>
<td>EV</td>
<td>62 ± 4</td>
<td>13 ± 2.0</td>
<td></td>
</tr>
</tbody>
</table>

* In this steroid competition assay, the ability of each analogue to compete with [3H]-1α,25(OH)2D3 was measured as described in "Materials and Methods"; the RCI of 1α,25(OH)2D3 is, by definition, 100.* When studying DBF, if [3H]-25(OH)D3 is used as a ligand, then the RCI for 25(OH)D3 is, by definition, 100 (see "Materials and Methods"). The RCI values for DBF have been normalized so that the apparent 1α,25(OH)2D3 RCI is set at KM. The actual RCI_{app} for 1α,25(OH)2D3 is 0.15, i.e., 1/666 that of 25(OH)D3.

![Graph A](image1.png)

![Graph B](image2.png)

Fig. 2. Dose-response effects of 1α,25(OH)2D3 and analogues V and EV on intestinal calcium absorption (A) and bone calcium mobilization (B) in vitamin D-deficient chicks. 1α,25(OH)2D3, analogue V, or analogue EV was given i.m. to vitamin D-deficient chicks 12 h before assay; the control vitamin D3 was given 48 h before assay. Results are expressed as the mean ± SE of groups of six or seven chicks. Each assay included a negative control (−D) and a positive control of vitamin D3 (3.25 nmol) (see "Materials and Methods"); the difference between these two groups was significant at P < 0.01.

in effecting a dose-dependent stimulation of both ICA and BCM; similar results have been repeated in other studies from this laboratory (16, 19).

Analogue EV, like 1α,25(OH)2D3, was able to produce a dose-dependent response of incremental increases for both ICA and BCM, although somewhat higher doses were required to obtain a maximum effect (Fig. 2). It can be estimated that analogue EV has approximately 20% of the activity of 1α,25(OH)2D3 in stimulating ICA and BCM. These calculations can be made by extrapolation of the dose of analogue EV required to produce a biological response equivalent to that of 100 pmol of 1α,25(OH)2D3. In contrast, analogue V yielded bioassay results indicating different responses for ICA and BCM. Thus, for BCM an incremental dose response was obtained (see Fig. 2B) over the dose range of 6.0–300 nmol, with significant stimulation above the vehicle control occurring at only 6.0 nmol. However, for ICA no evidence of an incremental dose response was obtained and the only stimulation of ICA occurred at the highest administered dose of 300 nmol. Considering that the ICA and BCM evaluations were carried out simultaneously in the same birds and that equivalent dose responses were obtained in these two assays with 1α,25(OH)2D3 and analogue EV, it is interesting that analogue V produced such different responses in these two target organs.

![Graph C](image3.png)

Fig. 3 reports the induction of calbindin-D28K in the intestinal mucosa (Fig. 3A) and kidney (Fig. 3B) at 24 h after i.m. dosing with either 1α,25(OH)2D3 or analogue V. Once again, evidence of a differential target organ response was noted, under conditions where both target organs were exposed simultaneously to the test substance. As anticipated, 1α,25(OH)2D3 was quite effective at stimulating the induction of calbindin-D28K in both target tissues; similar results have been reported previously (28). A reasonably linear log dose vs
calbindin-D_{28K} response was noted in both target tissues. In contrast, analogue V showed evidence of a calbindin-D_{28K} dose response only in the kidney. In the intestine, only the highest dose evaluated, 600 nmol of analogue V, induced calbindin-D_{28K}.

Fig. 4 summarizes results from the chick intestine and chick kidney regarding the occupancy of the VDR 1 h after i.m. administration of increasing doses of 1\alpha,25(OH)_{2}D_{3}, analogue V, or analogue EV. VDR occupancy is determined by in vitro incubation of the test tissue (intestinal or kidney) with increasing amounts of tritiated 1\alpha,25(OH)_{2}D_{3}, followed by separation of bound from free ligand using the standard hydroxylapatite procedure (22). Thus, when the tissue being examined was obtained from a vitamin D-deficient animal, there is maximal binding of \[^{3}H\]-1\alpha,25(OH)_{2}D_{3} in vitro, which is a measure of the total available unoccupied VDR at the time the tissue was harvested. Then, at varying time periods after administration of 1\alpha,25(OH)_{2}D_{3} or its analogues to the intact animal, followed by an in vitro assay, there are diminishing amounts of radioactive ligand bound, reflecting in vivo occupancy of the available VDR. In the determinations reported in Fig. 4 and subsequent figures, a formal Scatchard analysis was carried out on each sample. This allows a linear extrapolation of the \(B_{\text{max}}\), which is a measure of the total available receptor in that particular sample.

As shown in Fig. 4A, with increasing doses of 1\alpha,25(OH)_{2}D_{3} from 0.25 to 6.0 nmol there was quite a linear decrease in receptor occupancy; approximately 76% of the available receptor was occupied in vivo at the highest administered dose of 1\alpha,25(OH)_{2}D_{3}. Similarly, in the kidney (Fig. 4B) the highest dose of 1\alpha,25(OH)_{2}D_{3} administered occupied 87% of the available receptor.

For the two analogues of 1\alpha,25(OH)_{2}D_{3}, namely analogues EV and V, there was once again an apparent discrimination in the relative receptor occupancy between the intestine and the kidney, even though the concentrations of each ligand circulating in the plasma were identical for both target organs. When increasing doses of analogue EV were administered i.m., there was a uniform incremental increase in occupancy of the VDR in both the intestine (41%) (Fig. 4A) and the kidney (59%) (Fig. 4B). In contrast, when analogue V was administered i.m. in increasing doses, the intestinal VDR displayed resistance to receptor occupancy and only 10% occupancy was achieved at the highest dose of analogue V administered (150 nmol). In contrast, in the kidney there was an incremental increase in receptor occupancy with increasing doses of analogue V; the maximal receptor occupancy achieved was 43%.

Fig. 5 illustrates the relationship between occupancy of the VDR achieved 1 h after dosing with either 1\alpha,25(OH)_{2}D_{3} or analogue V and the induced level of calbindin-D_{28K} which was achieved by 24 h. Fig. 5A presents the results for the intestine, where it was apparent that 1\alpha,25(OH)_{2}D_{3} showed a reasonably linear relationship between occupancy of the receptor and subsequent induction of appearance of calbindin-D_{28K}. Similar results have been reported from this laboratory previously (28). Analogue V under the conditions tested showed no ability to induce calbindin-D_{28K}, nor could it achieve significant levels of occupancy of the intestinal VDR. In contrast, as shown in Fig. 5B, in the kidney both 1\alpha,25(OH)_{2}D_{3} and analogue V showed a linear relationship between VDR occupancy and the induction of calbindin-D_{28K}. These results clearly illustrate the difference in biological response achieved in intestine and kidney of the same animal.

The question then arose as to whether it was possible at any dose level to achieve significant intestinal receptor occupancy with analogue V. Also, it was pertinent to study the kinetics of VDR occupancy in the intestine and the kidney. Results of experiments which address these questions are presented in Fig. 6; here, the time course of VDR occupancy was determined after a single i.m. dose of 1\alpha,25(OH)_{2}D_{3} (6.0 nmol), analogue EV (30 nmol), or analogue V (300 nmol). Comparison of the results presented for the intestine (Fig. 6A) and the kidney (Fig. 6B) indicates that all three vitamin D compounds were able to achieve a significant occupancy of the VDR in both tissues at the dose levels tested. A notably rapid level of occupancy was achieved by 1 h, followed by 12–16 h of significant occupancy and then a decay over 24–48 h as the VDR lost its ligand and again became unoccupied. It is possible that the receptor occupancy values determined in the latter time interval (24–48 h) represent only approximations, since it is known that administration of 1\alpha,25(OH)_{2}D_{3} can effect up-regulation or down-regulation of VDR levels. Nonetheless, the important observation is that analogue V is able to achieve a significant duration of receptor occupancy in both intestine and kidneys when a sufficiently large dose is administered. In particular, there must not be any inherent tissue-specific blockade of the intestinal uptake of analogue V.

Fig. 7 reports the occupancy of the VDR which can be achieved in vivo in the intestine and the kidney when combined doses of 1\alpha,25(OH)_{2}D_{3} and analogue V are administered to vitamin D-deficient chicks. The purpose of this experiment was to address the question of whether moderate to high doses of analogue V would inhibit the uptake of low concentrations of 1\alpha,25(OH)_{2}D_{3} administered simultaneously. Thus, as shown in Fig. 7A, in the intestinal mucosa a single dose of 0.6 nmol of 1\alpha,25(OH)_{2}D_{3} achieved a receptor occupancy of 28% when 1\alpha,25(OH)_{2}D_{3} was administered alone. When 0.6 nmol of 1\alpha,25(OH)_{2}D_{3} was further supplemented with increasing doses of 6.0–600 nmol of analogue V, ultimately a VDR occupancy of 75% was achieved. This is the same receptor occupancy that was reported in Fig. 4 (intestine) when a high dose of 1\alpha,25(OH)_{2}D_{3} was administered. Similarly, as shown in Fig. 7B for the kidney, a single 0.6-nmol dose of 1\alpha,25(OH)_{2}D_{3} achieved a VDR occupancy of 57%.

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**Fig. 4.** 1\alpha,25(OH)_{2}D_{3} receptor occupancy in chick intestinal mucosa (A) and kidney (B) 1 h after i.m. administration of 1\alpha,25(OH)_{2}D_{3}, analogue V, or analogue EV. The quantitation of unoccupied VDR was as described in "Materials and Methods." For each point indicated, a Scatchard analysis was carried out and the extrapolated \(B_{\text{max}}\) was determined. The values are expressed as the mean ± SE of three separate determinations on tissue obtained from five separate birds. In the intestine (A) 100% = 229 ± 30 fmol/mg protein, while in the kidney (B) 100% = 38 ± 4 fmol/mg protein available as maximum unoccupied receptor binding.
DISCUSSION

This report is concerned with the integrated operation of the vitamin D endocrine system in the vitamin D-deficient chick and focuses on an evaluation of whether different target organs in the same animal display similar or selective stimulation of biological responses as a consequence of 1α,25(OH)₂D₃ or analogue administration. Analogues V and EV are both side-chain analogues of 1α,25(OH)₂D₃ (see Fig. 1). Analogue V has been extensively evaluated (16, 17, 29), while analogue EV has, to date, received only relatively minimal evaluation (20). In the context of this report it should be recognized that analogue V has been proposed for further consideration for clinical evaluation (17) as a consequence of its ability to promote cell differentiation without a calcemic effect. Analogue EV is included in the present study as a comparative side-chain analogue. In all the assays described in this report, 1α,25(OH)₂D₃ is the reference agonist.

As emphasized by a comparison of ICA versus BCM (Fig. 2) and by the relative occupancy of the VDR and the induction of calbindin-D₂₈K in both the intestine and kidney (Figs. 3–5), it is apparent that for analogue V there is discrimination in the intestine, compared with bone and kidney. Thus, while increasing doses of analogue V could produce incremental increases in VDR in the kidney, incremental increases in renal calbindin-D₂₈K, and an elevation in serum Ca²⁺ (a reflection of bone Ca²⁺ mobilization), there was virtually no intestinal uptake of analogue V. In comparison, for 22-oxa-1α,25(OH)₂-vitamin D₃, all three target organs (intestine, bone, and kidney) displayed incremental responses proportionate to the administered dose.

The relative ability of 1α,25(OH)₂D₃, analogue V, and analogue EV to bind in vitro to the chick intestinal VDR and plasma DBP was determined (see Table 1). With respect to binding to the intestinal VDR, the RCI of analogue V was 68% and that of analogue EV was 62%. Similar results were obtained for the kidney VDR (data not shown). These results imply that the classical nuclear receptor for 1α,25(OH)₂D₃ present in the chick intestine and kidney might bind analogues V and EV approximately equivalently under in vivo circumstances.

In terms of binding to the plasma DBP, [³H]-1α,25(OH)₂D₃ was used in this report as the reference compound, so its RCI was by
determinations. The results are the mean ± SE of individual determinations on four birds. The results are the mean ± SE of individual determinations on four birds.

While it is generally accepted that the bulk of biological responses initiated by 1α,25(OH)2D3 result from its association with the classical nuclear VDR, with concordant regulation of gene transcription (11, 13), there is the emerging view that some responses such as transcalcification, i.e., the "very rapid stimulation of intestinal Ca2+-absorption" (6, 27, 35), and the opening of Ca2+ channels (36) may occur by mechanisms that are not dependent upon the nuclear VDR. However, we have shown previously for 1α,25(OH)2D3 in the chick that there is a good correlation between the plasma level of 1α,25(OH)2D3 and the occupancy of the intestinal VDR, stimulation of CaBP, and ICA (28, 37). This correlation was made using the natural hormone 1α,25(OH)2D3; this secosteroid is the optimal agonist for both genomic and nongenomic responses. Here we have compared the relative abilities of 1α,25(OH)2D3 and analogues V and EV to affect the occupancy of the nuclear VDR (see Figs. 4 and 6). In both the intestine and kidney, increasing doses of 1α,25(OH)2D3 and analogue EV achieved, 1 h after dosing, proportionate occupancy of the VDR. Again, analogue EV was found to be only approximately 20% as effective as 1α,25(OH)2D3 in achieving 50% receptor occupancy. As emphasized in Figs. 4 and 5, doses of 6.0–150 nmol of analogue V were able to achieve in vivo occupancy of the kidney VDR and result in a proportionate increase in CaBPK, but in the intestine there was a marked inability of analogue V to achieve any occupancy of the VDR. These results reinforce the concept that there is a target organ-selective ability to achieve occupancy of the 1α,25(OH)2D3 nuclear VDR. But, as demonstrated in Fig. 6, when a very large dose (300 nmol) of analogue V was administered, as well as when a moderate dose (30 nmol) of analogue EV or high physiological levels (6 nmol) of 1α,25(OH)2D3 were administered, all three compounds were able to achieve a significant (up to 70%) and
sustained (up to 24 h) occupancy of both the intestinal and renal VDR. Thus, analogue V is able, when given in sufficiently large doses, to achieve occupancy of the intestinal VDR. Finally, as illustrated in Fig. 7, when combined doses of 1α,25(OH)2D3 and analogue V were given an intrinsic difference in occupancy response of the intestinal and kidney VDR was again apparent.

It is possible that differential target organ catabolism of an analogue may contribute to the generation of selective target organ responses; thus, the intestine may rapidly inactivate analogue V, leading to a blunting of the appearance of biological responses. Alternatively, the kidney may concentrate analogue V as a consequence of excretion processes; this could lead to a serendipitous increase in the renal concentrations of analogue V, leading to effective VDR occupancy and the induction of calbindin-D28k.

This report demonstrates that selective biological responses can be generated in 1α,25(OH)2D3 target organs in vivo in the same animal, under circumstances where the same ratio of free to bound analogue is present at all target tissues. This possibility was originally proposed 20 years ago by Hibberd and Norman (3); however, only recently has clear supportive evidence emerged. It is currently accepted that all target organ nuclear receptors for 1α,25(OH)2D3 are biochemically identical (13); in fact, the sequence of the human VDR present in HL-60 cells was found to be virtually identical to the sequence of the human chimeric (colon and T47D) VDR (38). As yet no published information exists concerning the chick VDR; however, there are no significant differences in the ligand-binding specificities between the rat, and human may exist with respect to responses to vitamin D analogues.

Thus, it would be expected that the intestine, bone, and kidney would respond similarly to all analogues; clearly, that is not the case for analogue V as evaluated in the present study. Indeed, the possibility exists that tissue-specific uptake of 1α,25(OH)2D3 analogues may result in generation of selective biological responses, although the biochemical basis for these effects is not presently known. In addition, there may be differences in target organ metabolism of analogues which result in more rapid inactivation of a given analogue in one target tissue, compared with another target tissue. However, now it does appear to be firmly established, from this report and previous studies (2, 4, 6, 16, 33), that analogues of 1α,25(OH)2D3 can be designed to produce selective biological responses in selected target tissues. Such analogues could be useful in treatment of certain forms of leukemia, psoriasis, and secondary hyperparathyroidism without inappropriate stimulation of hypercalcemia.

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