The Mechanism for the Disparate Actions of Calcitriol and 22-Oxacalcitriol in the Intestine*

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ABSTRACT

22-Oxacalcitriol (OCT) is one of several new analogs of vitamin D that retain many of the therapeutically useful properties of 1,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$], but have much less calcemic activity. In the present study we examined the actions of OCT on intestinal calcium absorption and calbindin D9k mRNA in vitamin D-deficient rats. After ip injection of OCT (1 μg/kg), calcium absorption increased significantly by 2 h and was maximal at 4 h (2.5-fold above control), but returned to pretreatment levels by 16 h. In contrast, the same dose of 1,25-(OH)$_2$D$_3$ caused a 5-fold increase in calcium absorption, which lasted more than 48 h. The transient effect of OCT on calcium absorption was also observed when the analog was infused at a dose of 1 μg/kg-day for 3 days. At the end of the infusion period, calcium absorption was 3-fold higher than that in vehicle-infused controls, but fell to pretreatment levels by 24 h after removal of the minipumps. The time courses for induction of calbindin D9k mRNA were similar for OCT and 1,25-(OH)$_2$D$_3$, with no change observed until more than 4 h after injection. However, calbindin mRNA levels returned to pretreatment values more rapidly in the OCT-treated rats. Consistent with these findings, we observed that a 1 μg/kg dose of [3H]OCT was completely cleared by 4-6 h after injection. This was paralleled by a loss of [3H]OCT associated with the intestinal vitamin D receptor. The rapid clearance of OCT is probably due to its low affinity for the serum vitamin D-binding protein. This low affinity would also be expected to allow greater accessibility to target cells. In support of this, we found that higher amounts of OCT than 1,25-(OH)$_2$D$_3$ were associated with the intestinal vitamin D receptor after the injection of several doses of these tritiated ligands. In summary, our results indicate that the pharmacokinetic properties of OCT are responsible at least in part for its low calcemic activity. Furthermore, comparison of the transient elevation of calcium absorption by OCT with its more prolonged effects on PTH and calbindin D9k indicates that each action of vitamin D compounds has a distinct biological half-life. The short circulatory half-life of OCT can exploit these differences to provide a therapeutic advantage in the treatment of vitamin D-responsive diseases. (Endocrinology 133: 1158–1164, 1993)

The primary function of vitamin D is to stimulate the intestinal absorption of calcium in response to hypocalcemic stress. This enhancement occurs mainly in the duodenum and is mediated by the activated form of the vitamin, 1,25-dihydroxyvitamin D$_3$ [1,25-(OH)$_2$D$_3$] (1–3). Three mechanisms for the absorption of calcium have been described. In the absence of vitamin D, calcium is absorbed mainly by a passive paracellular route, in which the rate of calcium absorption increases linearly with increasing luminal calcium. Vitamin D-dependent calcium transport is a transcellular process that can be saturated by high luminal calcium concentrations. When vitamin D-deficient animals are given 1,25-(OH)$_2$D$_3$, increases in calcium transport require several hours to become apparent. In contrast, using the isolated perfused duodenum technique, changes in calcium fluxes have been observed in vitamin D-replete animals within minutes after the introduction of 1,25-(OH)$_2$D$_3$ in the perfusate (4). This process, termed transcalcanchia, is not observed in vitamin D-deficient animals. It is likely that genomically encoded vitamin D-dependent proteins are required for the rapid nongenomic actions of 1,25-(OH)$_2$D$_3$ on calcium flux. A number of vitamin D-dependent proteins have been identified in the duodenum, including the well studied calbindin (5). The exact roles of these proteins in calcium movement are not fully understood.

Recent development of vitamin D analogs with selective activities has provided a new approach for studying the mechanism for vitamin D-dependent calcium transport. Several of these analogs retain the cell-differentiating activity of 1,25-(OH)$_2$D$_3$ but have very little calcemic activity, suggesting a resistance of bone and intestine to these compounds (6–10). The reason for the selective actions of these analogs is not clear. However, an important clue was provided by Okano and co-workers (11), who reported that one of these analogs, 22-oxa-1,25-(OH)$_2$D$_3$ (22-oxacalcitriol or OCT), had a very low affinity for the serum vitamin D-binding protein (DBP). This property is shared by the several other analogs with properties similar to those of OCT (12, 13). Furthermore, as predicted by its low affinity for DBP, OCT was found to be rapidly cleared in vivo and more accessible to target cells in culture (12). Despite its rapid clearance, OCT is as active as 1,25-(OH)$_2$D$_3$ in suppressing PTH synthesis in rats (14) and more active in enhancing the immune response in mice (15) and suppressing the growth of mammary carcinoma cells in mice (16), indicating an apparent tissue-specific response to this analog.

In the present study, we have directly examined the actions of OCT in the duodenum. Our results revealed that although OCT has a prolonged effect on calbindin D9k mRNA, it has
only a transient effect on intestinal calcium transport, consistent with its rapid clearance.

Materials and Methods

Materials

1,25-(OH)2D3 was generously provided by Dr. Milan Uskokovic, Hoffman LaRoche (Nutley, NJ). Radioinert OCT and [2-3H]OCT were supplied by Chugai Pharmaceutical Co., Ltd. (Tokyo, Japan). A cDNA probe for rat calbindin D9k was kindly provided by Dr. Hector DeLuca, Hoffman LaRoche (Arlington Heights, IL). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals

Male weanling Sprague-Dawley rats were maintained for 6 weeks on a vitamin D-deficient diet containing 0.4% calcium and 0.3% phosphorus (TD 86487, Teklad, Madison, WI). The rats were placed on a vitamin D-deficient, 0.02% calcium diet (Teklad TD 90407) for 2 days before treatment with the vitamin D compounds. At the time of the experiment, the rats weighed approximately 250 g. The total serum calcium level before treatment was 4.96 ± 0.29 mg/dL. Plasma 25-hydroxyvitamin D3 was undetectable (<0.3 ng/ml) in these rats, as assessed by HPLC purification and competitive binding assay. 1,25-(OH)2D3 levels were markedly reduced (27 ± 3 ng/ml to 40 to 60 pg/ml in normal rats). For the dose-response and time-course studies, OCT and 1,25-(OH)2D3 were administered ip in 200 μl propylene glycol. In another experiment, OCT and 1,25-(OH)2D3 were administered in propylene glycol at a dose of 1 μg/kg BW by sc infusion using osmotic minipumps (model 2002, Alzet Corp., Palo Alto, CA).

Intestinal calcium transport

The rate of intestinal calcium transport was measured at the specified time after treatment by a modification of the isolated duodenal loop method (17). The first 6 cm of the small intestine distal to the pyloric sphincter was flushed twice with 12 ml cold saline, rinsed off, and filled with 0.6 ml transport buffer (30 mM Tris-HCl [pH 7.4], 10 mM fructose, 125 mM NaCl, 10 mM CaCl2, and 5 μCi/ml 45Ca2+). After 10 min, the rats were exsanguinated, and the serum was analyzed for 45Ca2+. In this protocol, the appearance of 45Ca2+ in the blood increased linearly for at least 20 min, and therefore, the 10 min value represented a true rate of intestinal calcium absorption.

Assay of calbindin D9k mRNA

After the rats were exsanguinated, the duodenal mucosa was scraped and homogenized into 6 ml RNAzol (Cinna-Biotex, Friendswood, TX). RNA was prepared as described by the manufacturer. Total RNA (10 μg) was separated on a 1.2% agarose gel and transferred to a nylon membrane (MSI Magnagraph, Fisher Scientific, Pittsburgh, PA) by capillary action. RNA was cross-linked to the membranes by exposure to UV light (Stratalinker 2400, Stratagene, La Jolla, CA) and prehybridized in 7% sodium dodecyl sulfate (SDS), 0.5 M sodium phosphate (pH 7), and 1 mM EDTA at 65 g for 4 h. Random primer-labeled cDNAs for calbindin D9k and glyceraldehyde 3-phosphate dehydrogenase (GAPDH; 104 cpm/ml) were added, and hybridization was carried out at 65°C for 16 h. The membranes were washed twice at 65°C for 30 min with 5% SDS, 40 mM sodium phosphate (pH 7), and 1 mM EDTA and twice at 65°C for 30 min with 1% SDS in 40 mM sodium phosphate (pH 7.4) and 1 mM EDTA. The membranes were exposed to x-ray film (Hyperfilm MP, Amersham, Arlington Heights, IL), and the calbindin mRNA bands were quantified with a scanning densitometer (EC Apparatus Corp., St. Petersburg, FL).

Clearance of OCT and 1,25-(OH)2D3

Vitamin D-deficient rats were placed on the low calcium diet for 2 days and then injected ip with [2-3H]OCT or 1,25-(OH)2[26,27-3H]D3 (0.5, 1, or 2 μg/kg BW; 0.5 μCi/rat). At the specified times, the animals were killed by exsanguination under chloral hydrate anesthesia (360 mg/kg). Blood plasma (1 ml) was spiked with 250 ng radioinert OCT or 1,25-(OH)2D3 standard and extracted with C-18 silica cartridges by the method of Reinhardt et al. (18). The acetonitrile fraction was dried and analyzed by normal phase HPLC using hexane-isopropanol (92:8). Radioactivity eluting with standard OCT or 1,25-(OH)2D3 was determined, corrected for recovery based on the area of the UV peak of the standard, and expressed as picograms of OCT or 1,25-(OH)2D3 per ml serum.

In vivo labeling of intestinal vitamin D receptor (VDR)

The duodena of the rats injected with [2-3H]OCT or 1,25-(OH)2[26,27-3H]D3 were flushed twice with 12 ml cold saline containing soybean trypsin inhibitor (STI, 200 μg/ml) and slit lengthwise, and the mucosa was scraped with a chilled microscope slide. The mucosa was resuspended in 20 ml saline-STI and centrifuged at 200 x g for 10 min. This wash was repeated, and the mucosal pellet was homogenized in 10 ml TDE buffer (10 mM Tris-HCl [pH 7.4], 1.5 mM EDTA, 5 mM dithiothreitol, and 200 μg/ml STI). Nuclei were isolated by centrifugation at 500 x g for 10 min. The pellet was resuspended in 10 ml TDE containing 0.5% Triton X-100 and centrifuged at 5000 x g for 10 min. The Triton X-100 wash was repeated twice, and the final chromatin pellet was resuspended in 2 ml TDE containing 300 mM KC1 (final concentration). One milliliter was mixed with 9 ml ScintiVerse BD (Fisher Scientific) and counted for tritium in a Beckman LS-2800 scintillation counter (Palo Alto, CA). Another aliquot was analyzed for DNA (19). HPLC analysis of samples from rats treated with the 2 μg/kg dose of [2-3H]OCT or 1,25-(OH)2[26,27-3H]D3 was performed as described for the serum samples (see above) and confirmed that at least 75% of the tritium present in the sample coeluted with standard OCT or 1,25-(OH)2D3, respectively (data not shown).

To confirm that the [3H]OCT and 1,25-(OH)2[3H]D3 in these samples were bound to the VDR, sucrose gradient analysis was performed. The chromatin suspensions were centrifuged at 100,000 x g for 60 min, and an aliquot of the supernatant (200 μl) was layered over a 5–20% sucrose gradient in TDE-300 mM KC1. The gradients (15 ml total volume) were centrifuged at 40,000 rpm in an SW-40 rotor (Beckman Instruments) for 40 h. Fractions of 15 drops were collected from the bottom of the gradients, mixed with 4 ml ScintiVerse BD, and counted for tritium. The tritium peak in each sample was found to sediment just above the ovalbumin standard (3.7S), confirming that the [3H]OCT and 1,25-(OH)2[3H]D3 were bound to the duodenal VDR (data not shown).

Statistics

All data are expressed as the mean ± SEM, and experimental groups were compared by analysis of variance.

Results

We examined the time course of the action of OCT and 1,25-(OH)2D3 on intestinal calcium absorption. Vitamin D-deficient rats were injected with 1 μg/kg OCT or 1,25-(OH)2D3, and calcium transport was measured at 2, 4, 8, 16, 24, and 48 h later. Figure 1 shows that this dose of OCT stimulated calcium absorption significantly by 2 h (P < 0.01). Transport appeared maximal at 4 h (P < 0.05), although the transport rates at 2, 4, and 8 h were not different from each other. By 16 h after OCT injection, calcium absorption had returned to pretreatment levels. The slight increase at 24 h was not a consistent finding. In contrast, the increase in calcium transport after the same dose of 1,25-(OH)2D3 was delayed slightly and was much more prolonged. Calcium transport after 1,25-(OH)2D3 injection was not different from the control value at 2 h, but increased by 2.5-fold by 8 h, with a further increase of 3-fold by 24 h. With this dose of
Fig. 1. Time course of enhancement of intestinal calcium absorption by 1,25-(OH)₂D₃ or OCT. Vitamin D-deficient rats were injected ip with 1 µg/kg OCT or 1,25-(OH)₂D₃, and calcium transport was measured at various times thereafter. The data are expressed as a percentage of the control (vehicle-treated) value at each time point and are presented as the mean ± SEM (n = 6-10).

Fig. 2. Effect of constant infusion of 1,25-(OH)₂D₃ (1.25D in this and following figures) or OCT on intestinal calcium absorption. Vitamin D-deficient rats were infused with OCT or 1,25-(OH)₂D₃ at a rate of 1 µg/kg-day or with vehicle (propylene glycol). Intestinal calcium transport was measured after 3 days of infusion (■) or 24 h after the 3-day infusion was stopped (□). Data are presented as the mean ± SEM (n = 6).

1,25-(OH)₂D₃, maximal enhancement of calcium absorption was maintained for at least 48 h.

The transient stimulation of calcium transport by OCT was confirmed by infusing OCT with osmotic minipumps at a dose of 1 µg/kg-day. Three days of OCT infusion increased calcium uptake 3-fold compared to that in vehicle-infused controls (Fig. 2). This was similar to the increase achieved by infusion of the same dose of 1,25-(OH)₂D₃. Figure 2 also shows that calcium absorption dropped substantially in the OCT-infused rats, but remained elevated in the rats that had been infused with 1,25-(OH)₂D₃. These results confirm that the effects of OCT on calcium absorption are short-lived.

The time courses for the induction of calbindin D₉K mRNA by OCT and 1,25-(OH)₂D₃ (1 µg/kg) are shown in Fig. 3. Both OCT and 1,25-(OH)₂D₃ increased calbindin D₉K mRNA levels after 8 h. The data in Fig. 3 also show that the effect of OCT on calbindin mRNA is less sustained than that of 1,25-(OH)₂D₃. By 48 h after OCT injection, calbindin mRNA returned to pretreatment levels, whereas with the same dose of 1,25-(OH)₂D₃, calbindin mRNA was still elevated at 48 h.

The time courses of calcium transport after treatment were compared to the disappearance of OCT or 1,25-(OH)₂D₃ from the circulation. Vitamin D-deficient rats were injected ip with 1 µg/kg [³H]OCT or 1,25-(OH)₂[³H]D₃, and duodenal RNA was isolated at various times thereafter. Northern analysis was performed on 10 µg total RNA pooled from five rats at each time point. Bands corresponding to the mRNAs for calbindin and GAPDH in the autoradiograms shown were quantified by scanning densitometry and presented as a ratio of calbindin mRNA/GAPDH mRNA in the bar graph.

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The time course for binding of [³H]OCT and 1,25-(OH)₂-[³H]D₃ to the duodenal VDR of these rats is shown in Fig. 5. The time course of binding of each ligand closely parallels its serum levels. However, maximal levels of binding were much higher for OCT (3.5 pmol/mg DNA at 30 min) than for 1,25-(OH)₂D₃ (1.7 pmol/mg DNA at 2 h). Thus, despite the lower maximal levels achieved in the blood, OCT appeared to be more accessible to the intestinal receptor.

A dose-response study was performed using lower (0.5 µg/kg) and higher (2 µg/kg) doses of OCT and 1,25-(OH)₂D₃. [³H]OCT in plasma and associated with the duodenal VDR was measured 30 min postinjection. The same parameters were measured for 1,25-(OH)₂-[³H]D₃ after 2 h. These times
FIG. 4. Plasma levels of [3H]OCT and [3H]1,25-(OH)2D3. Vitamin D-deficient rats were injected ip with 1 μg/kg [3H]OCT or [3H]1,25-(OH)2D3 (2 μCi/μg). Blood was taken at the specified time, and an aliquot of plasma was extracted and analyzed by HPLC, as described in Materials and Methods. Values are the mean ± SD (n = 3).

FIG. 5. Time course of binding of [3H]OCT and [3H]1,25-(OH)2D3 to duodenal VDR. Vitamin D-deficient rats were injected ip with 1 μg/kg [3H]OCT or [3H]1,25-(OH)2D3 (2 μCi/μg). Chromatin extracts from duodenal mucosa were prepared and analyzed for [3H]OCT, [3H]1,25-(OH)2D3, and DNA, as described in Materials and Methods. The data are expressed as picomoles of OCT- or 1,25-(OH)2D3-associated VDR per mg DNA. Data are presented as the mean ± SD (n = 3).

FIG. 6. Plasma levels after increasing doses of [3H]OCT and [3H]1,25-(OH)2D3. Vitamin D-deficient rats were injected ip with 0.5, 1, or 2 μg/kg [3H]OCT or [3H]1,25-(OH)2D3. Plasma was analyzed for [3H]OCT after 30 min and for [3H]1,25-(OH)2D3 after 2 h. Data are presented as the mean ± SD (n = 3).

FIG. 7. Maximal VDR complex formation by various doses of [3H]OCT and [3H]1,25-(OH)2D3. Vitamin D-deficient rats were injected ip with 0.5, 1, or 2 μg/kg [3H]OCT or [3H]1,25-(OH)2D3. Chromatin extracts from duodenal mucosa were prepared and analyzed for [3H]OCT, [3H]1,25-(OH)2D3, and DNA, as described in Materials and Methods. The data are expressed as picomoles of OCT- or 1,25-(OH)2D3-associated VDR per mg DNA. Data are presented as the mean ± SD (n = 3).

were chosen to give maximal levels of binding for both ligands, maximal plasma levels of 1,25-(OH)2D3, and near-maximal plasma levels of OCT. The plasma levels of the two compounds are shown in Fig. 6. The levels of both OCT and 1,25-(OH)2D3 increased linearly with dose. A linear dose response was also observed for binding of both ligands to the duodenal VDR (Fig. 7). These data clearly show that OCT is much more accessible to the duodenal VDR.

Discussion

In the present study, we examined the calcemic actions of OCT in the intestine of vitamin D-deficient rats. We found that OCT is capable of stimulating calcium absorption, but this effect is only transient. In contrast, the same dose of 1,25-(OH)2D3 caused a prolonged elevation of calcium transport. The different responses of these two vitamin D compounds are probably related to their very different pharmacokinetics. As shown in Fig. 4, by 4 h after injection of a 1 μg/kg dose, nearly all of the OCT had been cleared. Although much of this clearance is due to rapid excretion of OCT metabolites in the bile (Nishii, Y., personal communication), uptake into target tissues also contributes to the disappearance from the circulation. However, the loss of OCT from the blood was paralleled by a loss of receptor-associated OCT in the duodenum. The enhanced calcium transport began to return to pretreatment levels after that time. It is likely that the slower disappearance of 1,25-(OH)2D3 from the circulation results in its more prolonged action on calcium transport, but additional factors may also be involved.

A pharmacokinetic explanation for the disparate actions of OCT and 1,25-(OH)2D3 in the intestine would not be unexpected considering the previously reported low affinity of the analog for the serum DBP (11, 12). This low affinity leads to rapid clearance of OCT and very likely limits the
duration of its effect on calcium transport. Another consequence of low DBP affinity would be a higher proportion of OCT compared to 1,25-(OH)\textsubscript{2}D\textsubscript{3} in the unbound form. Several investigators have shown that DBP (or whole serum) limits access of 1,25-(OH)\textsubscript{2}D\textsubscript{3} to cells in culture (20, 21). In the present study, we found that an analog of 1,25-(OH)\textsubscript{2}D\textsubscript{3} with lower affinity for DBP is more accessible to target cells in vivo. Higher amounts of OCT were associated with intestinal VDR despite the lower peak levels in the blood and a lower affinity for the VDR (measured in vitro). These data provide in vivo data to support a role for DBP in limiting the cellular uptake and action of 1,25-(OH)\textsubscript{2}D\textsubscript{3}. In addition, the lower DBP affinity and higher percent free OCT may explain its more rapid effect on calcium absorption. The rapid fall in intestinal calcium absorption after OCT clearance indicated the existence of a short-lived factor(s) that is essential for maintaining the increased rate of calcium transport. Calbindin D9k does not appear to be this factor. After OCT administration, the levels of calbindin D9k mRNA remained elevated when calcium transport returned to control levels. The role of calbindin in intestinal calcium absorption is not well defined. Attempts have been made to assign a role for calbindin on the basis of its temporal relationship with calcium absorption after treatment with 1,25-(OH)\textsubscript{2}D\textsubscript{3}. Although several groups have found that calbindin levels increase before or at the same times as calcium absorption (22–25), others (26–28) have observed that calbindin increases after calcium transport is stimulated. Under our conditions, calbindin D9k did not appear to increase until after calcium transport was elevated. However, Dupret et al. (25) observed a transient increase in calbindin D9k within 15 min after ip administration of 1,25-(OH)\textsubscript{2}D\textsubscript{3} to vitamin D-deficient rats. The spike in calbindin D9k mRNA may play a role in the increased calcium absorption within 2 h of OCT administration.

Dissociation of calbindin induction and calcium absorption using vitamin D analogs was also reported by Krisinger et al. (29). In contrast to our results with OCT, they found that side-chain homologs of 1,25-(OH)\textsubscript{2}D\textsubscript{3} could induce calbindin D9k mRNA with not even a transient effect on intestinal calcium transport. It is unclear whether this represents distinctively different properties of these 24-homo analogs, a dose insufficient to have any effect on transport, or an extremely rapid clearance of these analogs. These data obtained with vitamin D analogs provide new insights into the role of calbindin D9k in the mechanism of 1,25-(OH)\textsubscript{2}D\textsubscript{3}-stimulated calcium absorption.

The much more prolonged effect of OCT on calbindin, compared to that on calcium transport, is noteworthy. We have found that other genomic actions of OCT have similar time courses. OCT is capable of suppressing 1,25-(OH)\textsubscript{2}D\textsubscript{3} synthesis and inducing its degradation (29). Despite the rapid clearance of OCT, serum 1,25-(OH)\textsubscript{2}D\textsubscript{3} levels and renal 1α-hydroxylase activity remained suppressed for 2–3 days after administration (30, 31). The inhibition of 1,25-(OH)\textsubscript{2}D\textsubscript{3} by OCT may result in part from the suppressive action of the analog on PTH secretion. We found that PTH levels in uremic dogs remain below pretreatment levels for 2–3 days after a single injection of OCT (10). Thus, the time course for induction of calbindin is similar to that for other genomic actions of OCT. It is the rapid recovery of calcium transport to pretreatment levels that is unique.

Our time course for calcium transport after 1,25-(OH)\textsubscript{2}D\textsubscript{3} treatment differs from the biphasic response reported by Halloran and DeLuca (32). These researchers observed an initial peak of transport at 6 h, followed by a nadir at 12 h and a second increase to a plateau at 24 h. In their study, Halloran and DeLuca administered 1,25-(OH)\textsubscript{2}D\textsubscript{3} iv, whereas in the present study, the vitamin D compounds were administered ip. The slow absorption of 1,25-(OH)\textsubscript{2}D\textsubscript{3} from the peritoneal cavity in our experiments probably prevented resolution of the two processes. The later prolonged phase observed by Halloran and DeLuca was proposed to be due to migration up the villi of cells that had been in the villus crypt at the time of 1,25-(OH)\textsubscript{2}D\textsubscript{3} administration. The time course of calcium transport after OCT administration gave only a single peak at 4–8 h. It is possible that OCT is unable to support this second, more prolonged phase. Further experiments will be necessary to determine the differentiating activity of OCT in the intestine.

The results of this study support the hypothesis that pharmacokinetics are responsible for the very low calcemic activity of OCT and possibly the other noncalcemic vitamin D analogs. However, other explanations cannot be ruled out. Recent reports have indicated that OCT and other analogs with properties similar to those of OCT (i.e., high differentiating activity, low calcemic activity, high affinity for the VDR, and low affinity for DBP) may be unable to support the nongenomic actions of 1,25-(OH)\textsubscript{2}D\textsubscript{3}. OCT has been shown to be incapable of mimicking the ability of 1,25-(OH)\textsubscript{2}D\textsubscript{3} to increase cytosolic calcium levels in ROS 17/2.8 cells (33). In addition, several other analogs of 1,25-(OH)\textsubscript{2}D\textsubscript{3} with modifications in the side-chain and D-ring were reported to have minimal effect on cytosolic calcium in ROS 17/2.8 cells (34).

1,25-(OH)\textsubscript{2}D\textsubscript{3} has been shown to trigger a rapid influx of calcium through a voltage-regulated calcium channel on the basolateral surface of enterocytes (35, 36). Other agents that stimulate calcium influx through this channel also increase the transcalcachia pathway (37). Several vitamin D analogs with low calcemic activity have been shown to be relatively inactive in stimulating transcalcachia (37). On the other hand, several analogs of 25-hydroxyvitamin D that have poor affinity for the nuclear VDR were active in stimulating intracellular calcium (34) and transcalcachia (37). These data suggest the existence of a second VDR that is responsible for the rapid nongenomic actions of vitamin D compounds. The actions of OCT on cytosolic calcium in the enterocyte and transcalcachia have not been reported.

Other mechanisms for the selectivity of noncalcemic analogs are also possible. Multiple forms of the VDR could exist, and some of these may not recognize various analogs of 1,25-(OH)\textsubscript{2}D\textsubscript{3}. Little is known about the in vivo metabolism of these analogs. Tissue-specific catabolism or activation could play a role in their selectivity.

In summary, our results show the following. 1) OCT is
capable of stimulating intestinal calcium transport, but this effect is short-lived. This finding is consistent with the rapid clearance of OCT from the circulation and the ensuing loss of OCT-VDR complexes. 2) Calbindin D9K mRNA does not increase following OCT treatment until after calcium transport is maximal and remains elevated after calcium transport returns to pretreatment levels. 3) The low affinity of OCT for serum DBP not only results in rapid clearance of this analog, but also makes it more accessible to target tissues. 4) The rate of calcium transport returns to pretreatment levels with a few hours after OCT disappears from the circulation. This differs from the more prolonged effects, such as induction of calbindin D9K mRNA and 24-hydroxylation activity and suppression of PTH secretion and lcr-hydroxylase activation of calbindin D9K mRNA and 24-hydroxylase activity. This differs from the more prolonged effects, such as induction of calbindin D9K mRNA and 24-hydroxylation activity and suppression of PTH secretion and lcr-hydroxylase activation of calbindin D9K mRNA and 24-hydroxylase activity.

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References

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36. de Boland AR, Norman AW 1990 Influx of extracellular calcium mediates 1,25-dihydroxyvitamin D3-dependent transcaltachia (the rapid stimulation of duodenal Ca2+ transport). Endocrinology 127:2475–2480