Journal of Cellular Biochemistry 73:106–113 (1999)

1α,25-Dihydroxy-3-Epi-Vitamin D_3, a Natural Metabolite of 1α,25-Dihydroxyvitamin D_3, Is a Potent Suppressor of Parathyroid Hormone Secretion

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Abstract 1α,25$(OH)_2D_3$ is an important negative regulator of parathyroid hormone (PTH) gene transcription. In parathyroid cells, as in other target tissues, 1α,25$(OH)_2D_3$ is degraded by side chain oxidation by the inducible 24-hydroxylase. We have previously shown that one metabolite of this pathway, 1α,23(S),25-$(OH)_3$-24-oxo-D_3, potently suppresses PTH synthesis and secretion in cultured bovine parathyroid cells (bPTC). Further examination of the metabolites of 1α,25$(OH)_2D_3$ in bPTC has revealed another compound that is less polar than 1α,25$(OH)_2D_3$. By HPLC analysis and mass spectrometry, this metabolite was identified as 1α,25$(OH)_2$-3-epi-D_3. The activity of this metabolite on PTH gene transcription was assessed by the steady-state PTH secretion by bPTC after 72-h treatment with concentrations from 10^{-11} M to 10^{-7} M. 1α,25$(OH)_2$-3-epi-D_3 was found to be only slightly, but not significantly, less active than the native 1α,25$(OH)_2D_3$ in suppressing PTH secretion despite having 30 times lower affinity for the bPTC VDR. Both 1α,25$(OH)_2D_3$ and 1α,25$(OH)_2$-3-epi-D_3 maximally suppressed PTH secretion by 50%. Along with 1α,25$(OH)_2$-3-epi-D_3, the activities of the other two A-ring diastereomers were assessed. 1β,25$(OH)_2D_3$ suppressed PTH only at 10^{-7} M with a decrease of only 30%, in good agreement with its low VDR affinity. Surprisingly, 1β,25$(OH)_2$-3-epi-D_3 stimulated PTH secretion by 30–50% at concentrations from 10^{-11} M to 10^{-8}M and fell to control (untreated) rates at 10^{-7}M. The mechanism for this increase in PTH secretion is under investigation. Metabolism studies performed in bPTC cells using high concentrations of 1α,25$(OH)_2D_3$ substrate showed that in some incubations, the concentration of 1α,25$(OH)_2$-3-epi-D_3 was even higher than that of the parent substrate 1α,25$(OH)_2D_3$. This finding indicates a slower rate of metabolism for this diastereomer. Thus, production and accumulation of 1α,25$(OH)_2$-3-epi-D_3, as a major stable metabolite of 1α,25$(OH)_2D_3$ in parathyroid glands, may contribute to the prolonged suppressive effect of 1α,25$(OH)_2D_3$ on PTH gene transcription. J. Cell. Biochem. 73:106–113, 1999.

Key words: 1α,25$(OH)_2D_3$; PTH regulation; gene transcription

The suppressive action of vitamin D on parathyroid hormone (PTH) secretion is an integral regulatory component of calcium homeostasis. 1α,25-Dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], the active, hormonal form of vitamin D_3, regulates PTH synthesis and secretion by several mechanisms. Silver et al. [1986] and Russell et al. [1986] demonstrated that 1α,25-$(OH)_2D_3$ inhibits PTH gene transcription. This action is mediated by the vitamin D receptor present in the parathyroid glands [Brumbaugh et al., 1975; Henry and Norman, 1975], and 1α,25-$(OH)_2D_3$-responsive negative regulatory elements have been identified in the human [Demay et al., 1992] and chick [Liu et al., 1996] PTH gene promoters. In addition to this direct effect on PTH, 1α,25-$(OH)_2D_3$ has been shown to increase the sensitivity of the parathyroid glands to the suppressive action of calcium [Brown et al., 1996; Delmez et al., 1989] and block parathyroid gland hyperplasia in uremic rats [Szabo et al., 1989].

Another action of 1α,25$(OH)_2D_3$ in parathyroid cells and in other target cells is the induction of metabolic pathways that oxidize the side chain of vitamin D compounds, including 1α,25$(OH)_2D_3$ itself [Brown et al., 1992; Jones et al., 1987; Ohnuma et al., 1980; Reddy and

Contract grant sponsor: National Institute of Diabetes and Digestive Diseases; Contract grant numbers: DK-009976, DK-30178, DK-07126.

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Received 2 September 1998; Accepted 8 October 1998

Tserng, 1989; Reddy et al., 1987]. While the side chain oxidation is generally believed to play an attenuating role by degrading $1\alpha,25(OH)_2D_3$, it is clear that some metabolites of $1\alpha,25(OH)_2D_3$ retain significant biological activity [Ishizuka et al., 1990; Lee et al., 1997]. We have recently reported that one metabolite of the side chain oxidation pathway, 1α,23(S),25-trihydroxy-24-oxo-vitamin D_3, is nearly as active as $1\alpha,25(OH)_2D_3$ in suppressing PTH secretion [Lee et al., 1997]. The activities of other metabolites of $1\alpha,25(OH)_2D_3$ have not been investigated.

In the present study, we have continued our examination of the products of $1\alpha,25(OH)_2D_3$ metabolism in parathyroid cells. We report here the isolation of a major, less polar metabolite that we have identified by high-performance liquid chromatography (HPLC) and mass spectrometry as $1\alpha,25(OH)_2$-3-epi-D_3. This diastereomer of $1\alpha,25(OH)_2D_3$ is as active as $1\alpha,25(OH)_2D_3$ in suppressing PTH secretion. In addition, we have examined the activities of the other two diastereomers of $1\alpha,25(OH)_2D_3$.

MATERIALS AND METHODS

Materials

The $1\alpha,25$-$(OH)_2$-[26,27-^{3}H]vitamin D_3, specific activity 160–180 Ci/mmol was obtained from Amersham. Bovine serum albumin (BSA) was fraction V (Sigma, St. Louis, MO). Unlabeled $1\alpha,25(OH)_2D_3$ was a gift from Dr. Milan Uskokovic of Hoffmann LaRoche (Nutley, NJ). A-ring diastereomers of $1\alpha,25(OH)_2D_3$ were synthesized as described previously [Muralidharan et al., 1993]. All the natural metabolites of $1\alpha,25(OH)_2D_3$ used as standards were produced enzymatically using the isolated kidney perfusion system as described before [Reddy and Tserng, 1989; Reddy et al., 1987]. All other chemicals and solvents were of the highest grade available.

High Performance Liquid Chromatography and Mass Spectrometry

HPLC was performed with a Waters System Controller (model 600E) equipped with a photodiode detector (model PDA 990) to monitor ultraviolet (UV) absorbing material at 265 nm (Waters Associates, Milford, MA). All solvents for HPLC were obtained from Burdick and Jackson Laboratories, Muskegan, MI. Mass spectra (70 eV) were obtained on a Hewlett-Packard 5985B mass spectrometer by introducing samples of metabolites (0.5–1.0 µg) into the ion source maintained at 200°C via a direct-insertion probe.

Cell Culture

Bovine parathyroid glands were obtained from a local slaughterhouse and transported to the laboratory in cold phosphate-buffered saline (PBS). The glands were digested with collagenase as previously described [Brown et al., 1992] and seeded at a density of 80,000 cells/cm^2 in DMEM-Ham's F-12 (1:1) containing 4% heat-inactivated newborn calf serum, 15 mM HEPES, 100 IU/ml penicillin, 100 µg/ml streptomycin, 5 µg/ml insulin, 2 mM glutamine, 5 µg/ml holo-transferrin, and 1% nonessential amino acids. After 24 h, cells were placed in medium containing 0.1% BSA in place of the serum. Except for the initial 24 h, the cells were grown to confluency (6 days) in serum-free medium.

Analysis of $1\alpha,25(OH)_2D_3$ Metabolites

Confluent cultures of parathyroid cells were treated overnight with 10 nM $1\alpha,25(OH)_2D_3$ in serum-free medium to induce the metabolic pathway(s). The cells were then incubated with 1 µM $1\alpha,25(OH)_2D_3$ in serum-free medium for 24 h. Incubations were terminated by the addition of an equal volume of methanol containing 25-hydroxyvitamin D_3 as an internal standard to correct for recovery. Lipid extraction of both medium and cells was performed using the procedure described previously [Siu-Caldera et al., 1996]. The lipid extract was analyzed by straight-phase HPLC using a Zorbax-Sil column (4.6 mm × 25 cm) eluted with four different solvent mixtures at a flow rate of 2 ml/min. HPLC analysis of the lipid extracts was first conducted using a solvent mixture consisting of 10% isopropanol in hexane (solvent mixture no. 1). Subsequently, a second solvent mixture consisting of 6% isopropanol in hexane (solvent mixture no. 2) was used to obtain a better resolution of the metabolism products. Finally, purification of the metabolites for mass spectrometric analysis was obtained using a Zorbax-Sil column eluted with two different solvent mixtures, namely, 4% isopropanol in methylene chloride (solvent mixture no. 3) and 12% isopropanol in hexane (solvent mixture no. 4).

Analysis of PTH Secretion

Parathyroid cell cultures were prepared as described above. On the third day of culture the cells were treated with the vitamin D compounds (1α,25$(OH)_2D_3$ or its diastereomers) at concentrations ranging from 10 pM to 100 nM with daily changes of the medium for 3 days. Steady-state PTH secretion was determined by washing the cells three times with Dulbecco's PBS and then placing them in treatment media for 3 h. The media were collected, centrifuged at 4°C and analyzed for PTH using CH9 antibody as described previously [Hruska et al., 1975]. The cell monolayers were dissolved in 0.1 N NaOH and assayed for protein by the method of Bradford [Bradford, 1976] using a kit from Bio-Rad Laboratories (Richmond, CA). PTH secretion is expressed as ng PTH per mg cell protein. A two-tailed *t*-test was used to analyze the data.

Induction of 1α,25$(OH)_2D_3$ Metabolism by 1α,25$(OH)_2D_3$ and Its Diastereomers

Parathyroid cells were grown to confluency in 12-well plates. 1α,25$(OH)_2D_3$ or its diastereomers were added at the specified concentration. After 24 h, the cells were washed twice with fresh medium and incubated with 200 µl of medium containing 5 nM 1α,25-$(OH)_2$-[26,27-^{3}H]D_3 (17 Ci/mmol) for 30 min. Metabolism of tritiated 1α,25$(OH)_2D_3$ was assessed during incubation with intact parathyroid cells by extracting the cells and medium together. The incubations were stopped by addition of one volume of acetonitrile to the culture. Unlabeled 1α,25$(OH)_2D_3$ (250 ng) was added as internal standard to determine recoveries. The cell debris was scraped from the culture dish and the samples were transferred to borosilicate tubes. After centrifugation to remove precipitated protein, the samples were applied to C18 silica cartridges (Fisher Scientific, Pittsburgh, PA). The cartridges were washed with 5 ml of water, and the 1α,25$(OH)_2D_3$ was eluted with 5 ml of acetonitrile. The 1α,25$(OH)_2D_3$ fractions were resolved by normal phase HPLC (ZorbaxSil) using hexane:isopropanol:methanol (88:11:1). The 1α,25$(OH)_2D_3$ fractions were collected, mixed with ScintiVerse BD and counted. Final values were corrected for recovery as assessed by the UV absorbance of the internal standard.

Determination of Vitamin D Receptor Affinity in Intact Cells

Confluent parathyroid cells were incubated for 2 h with 0.5 nM 1α,25-$(OH)_2$-[26,27-^{3}H]D_3 and the indicated concentration of radioinert 1α,25$(OH)_2D_3$ or diastereomer in serum-free medium containing 0.5 mg BSA/ml. The medium was aspirated and the cells were washed once with PBS containing 5 mg/ml BSA and then twice more with ice-cold PBS. The cells were sonicated in cold TEDK buffer (10 mM Tris-HCl, pH 7.4, 1.5 mM EDTA, 5 mM dithiothreitol, 300 mM KCl), and an aliquot was taken to determine total cell protein. The remaining sonicate was mixed with 200 µl of charcoal/dextran, kept on ice for 15 min, and centrifuged at 1,000*g* for 15 min. The supernatants were mixed with 4 ml of ScintiVerse BD (Fisher Scientific, Pittsburgh, PA) and counted in a Beckman LS-2800 liquid scintillation counter. The data are expressed as fmol of [^{3}H]-1α,25$(OH)_2D_3$ bound per mg cell protein.

RESULTS

Identification of Metabolites of 1α,25$(OH)_2D_3$

The metabolism of 1α,25$(OH)_2D_3$ was examined in confluent bovine parathyroid cells pretreated for 24 h with 10 nM 1α,25$(OH)_2D_3$. This pretreatment induced the metabolic enzymes for 1α,25$(OH)_2D_3$. The cells were then incubated for 24 h with 1 µM 1α,25$(OH)_2D_3$ and the metabolites were extracted as described under Materials and Methods. The incubations were repeated three times. Control incubations without cells indicated no metabolism of 1α,25$(OH)_2D_3$. Analysis by straight-phase HPLC showed several peaks that coeluted with authentic standards of 1α,24(R),25-$(OH)_3D_3$, 1α,25-$(OH)_2$-24-oxo-D_3, 1α,23(S),25-$(OH)_3$-24-oxo-D_3, 1α,23-$(OH)_2$-tetranor-D_3 (C-23 alcohol) and 1α,23(S),25$(OH)_3D_3$ (Fig. 1). Further evidence for the structures of these metabolites was obtained using the diode array detector used in this HPLC system. Each peak had the distinctive ultraviolet spectrum of vitamin D compounds with a lambda max at 264 nm and a minimum at 228 nm. Final confirmation of each metabolite was achieved through mass spectrometry (data not shown).

In addition to products of the C-24 and C-23 oxidation pathways, a less polar metabolite was observed that eluted just before 1α,25$(OH)_2D_3$ and coeluted with authentic standard for

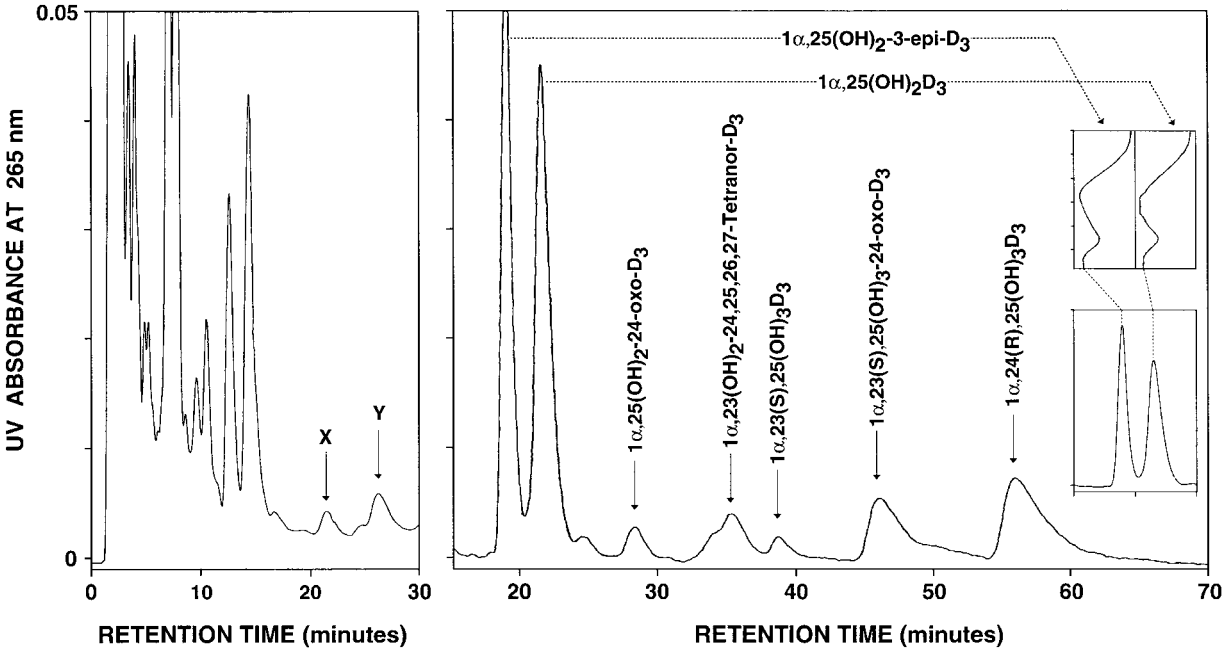

Fig. 1. High-performance liquid chromatography (HPLC) profile of the metabolites of 1α,25-(OH)$_2$D$_3$ produced by cultured bovine parathyroid cells. Parathyroid cells were treated for 16 h with 10 nM 1α,25-(OH)$_2$D$_3$ and then for 24 h with 1 μM 1α,25-(OH)$_2$D$_3$. Lipid extract of both cells and medium was analyzed by normal-phase HPLC, using two solvent mixtures as described under Materials and Methods. Left panel: HPLC profile of 1α,25(OH)$_2$D$_3$ and its metabolites analyzed, using solvent mixture no. 1. Peaks X and Y co-eluted with 1α,25(OH)$_2$D$_3$-lactone and 1α,25(R),26(OH)$_3$D$_3$, respectively. No further attempt was made to identify these peaks. Right panel: HPLC profile of 1α,25(OH)$_2$D$_3$ and its metabolites eluting at 5–20 min of the first HPLC separation (left panel) were re-analyzed using solvent mixture no. 2. Inset, UV spectra of the substrate and the less polar metabolite, later identified as 1α,25(OH)$_2$-3-epi-D$_3$.

1α,25(OH)$_2$-3-epi-D$_3$. The identity of this metabolite as 1α,25(OH)$_2$-3-epi-D$_3$ was established by mass spectrometry and its co-elution with synthetic standard of 1α,25(OH)$_2$-3-epi-D$_3$ on both normal and reverse-phase HPLC systems. The mass spectrum of the metabolite exhibited a molecular ion M/Z 416 and mass fragmentation pattern identical to the substrate 1α,25 (OH)$_2$D$_3$ (data not shown). This finding indicated that the metabolite produced in parathyroid cells is indeed an isomer of 1α,25(OH)$_2$D$_3$; therefore, it had to be one of the three possible A-ring diastereomers. The retention times of 1α,25(OH)$_2$D$_3$ and its three A-ring diastereomers along with the metabolites produced in parathyroid cells are given in Table I. Straight-phase HPLC was performed using a Zorbax-Sil column (4.6 mm × 25 cm) eluted with 6% isopropanol in hexane at a flow rate of 2 ml/min. Reverse-phase HPLC was performed using a Zorbax-ODS column (4.6 mm × 25 cm) eluted with 20% water in methanol. The metabolite co-migrated on both normal and reverse-phase HPLC with 1α,25(OH)$_2$-3-epi-D$_3$, confirming unequivocally that the metabolite in bovine parathyroid cells was 1α,25(OH)$_2$-3-epi-D$_3$.

TABLE I. Identification of the Less Polar Metabolite of 1α,25(OH)$_2$D$_3$ Produced in the Bovine Parathyroid Cells by Straight and Reverse-Phase HPLC Systems[a]

	Retention time	
Vitamin D$_3$ compounds	Straight-phase HPLC (min)	Reverse-phase HPLC (min)
1α,25(OH)$_2$D$_3$	21.36	22.51
1β,25(OH)$_2$-3-epi-D$_3$	21.87	22.58
1β,25(OH)$_2$D$_3$	19.52	23.24
1α,25(OH)$_2$-3-epi-D$_3$	18.84	21.44
Less polar metabolite	18.82	21.41

[a]HPLC, high-performance liquid chromatography.

Biological Activities of 3-epi-1α,25(OH)$_2$D$_3$

The biological activity of 1α,25(OH)$_2$-3-epi-D$_3$ in parathyroid cells was determined by comparing the potency of this metabolite to that of 1α,25(OH)$_2$D$_3$ in two genomic responses: suppression of PTH and induction of 1α,25(OH)$_2$D$_3$

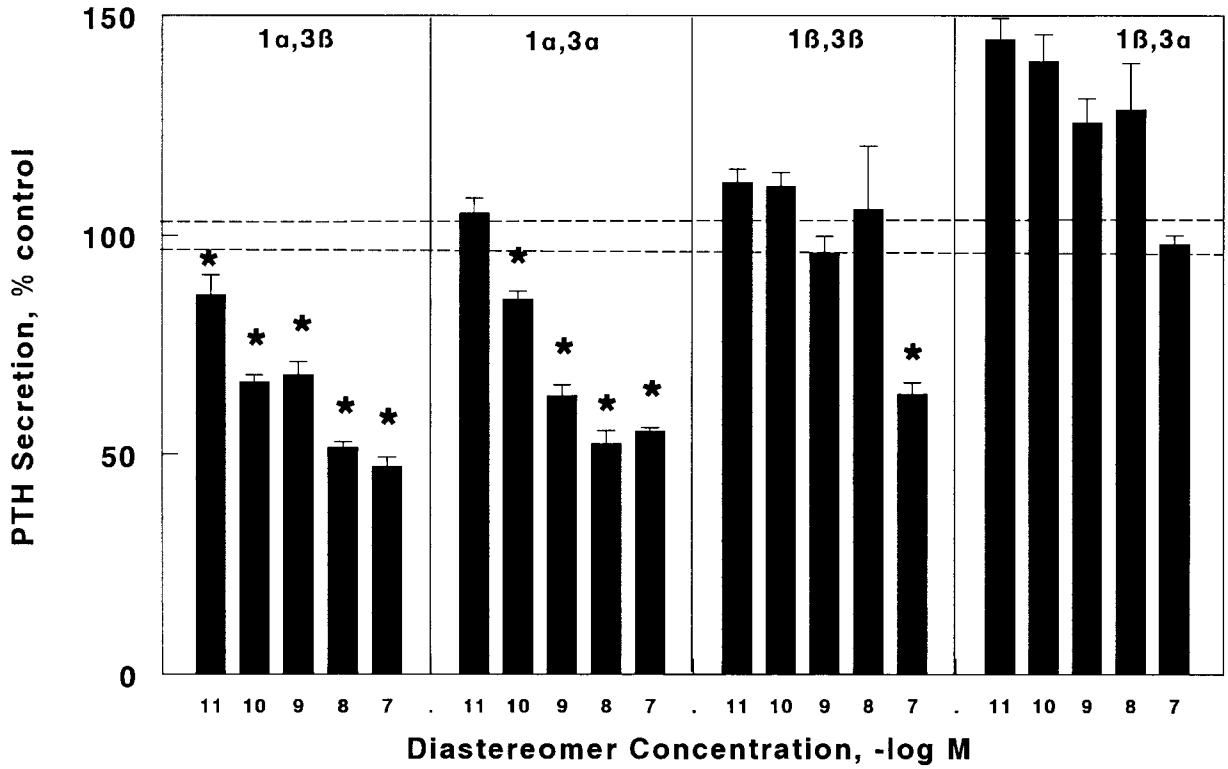

Fig. 2. Suppression of PTH secretion by A-ring diastereomers. Bovine parathyroid cells were incubated with the specified concentration of 1α,25-$(OH)_2D_3$ (1α,3β), 1α,25$(OH)_2$-3-epi-D_3 (1α,3α), 1β,25$(OH)_2D_3$ (1β,3β), or 1β,25$(OH)_2$-3-epi-D_3 (1β,3α) for 72 h with media changes every 24 h. The cells were then washed and steady-state PTH secretion was determined during a 3-h incubation. PTH was assessed by radioimmunoassay, and data were corrected for total cell protein. The secretion rates are given as percentage of the untreated control and represent combined data from two separate experiments (n = 12).

metabolism. To assess the effects on PTH secretion, parathyroid cells were incubated with various concentrations of 1α,25$(OH)_2D_3$ or 1α,25$(OH)_2$-3-epi-D_3 for 3 days. The steady-state rate of PTH secretion was then measured during a 3-h period. As shown in Figure 2, 1α,25$(OH)_2$-3-epi-D_3 potently decreased PTH secretion. This suppression was significant ($p < 0.01$) at 10^{-10} M. While 1α,25$(OH)_2$-3-epi-D_3 appeared to be slightly less active than 1α,25$(OH)_2D_3$, the difference was not significant.1α,25$(OH)_2$-3-epi-D_3 also actively induced vitamin D metabolism, although it was 10 times less active than 1α,25$(OH)_2D_3$ (Fig. 3).

These relatively high activities of 3-epi-1α,25$(OH)_2D_3$ correlates well with the reported affinity for the VDR of chick intestine [Bouillon et al., 1995]. VDR affinity for mammalian VDR has not been reported. Therefore, we determined the accessibility of 1α,25$(OH)_2$-3-epi-D_3 to the vitamin D receptor in monolayers of parathyroid cells using a competitive binding assay. Figure 4 shows that 1α,25$(OH)_2$-3-epi-D_3 was approximately 30 times less effective than radioinert 1α,25$(OH)_2D_3$ in blocking the uptake and receptor binding of [^{3}H]-1α,25$(OH)_2D_3$ in parathyroid cells.

Biological Activities of A-Ring Diastereomers

To further explore the effects of epimerization of the A-ring hydroxyl groups, we also examined the activities of carbon 1 diastereomers. Figure 2 shows the PTH suppression by 1α,25$(OH)_2D_3$(1α,3β),1α,25$(OH)_2$-3-epi-D_3 (1α, 3α), 1β,25$(OH)_2D_3$ (1β,3β) and 1β,25$(OH)_2$-3-epi- D_3(1β,3α). 1β,25$(OH)_2D_3$ was inactive except at 10^{-7} M where it produced about a 30% reduction in PTH secretion. 1β,25$(OH)_2$-3-epi-D_3, containing the unnatural stereochemistry (compared with native 1α,25$(OH)_2D_3$) at both carbons 1 and 3, elicited a surprising 30–50% *increase* in PTH secretion rate at low concentrations with a drop back to control rates at 10^{-7} M. Induction of 1α,25$(OH)_2D_3$ metabolism by the A-ring diastereomers was also measured (Fig. 3). 1β,25$(OH)_2D_3$ induced metabolism significantly at 10^{-9} M, while 1β,25$(OH)_2$-3-epi-D_3 showed very little activity.

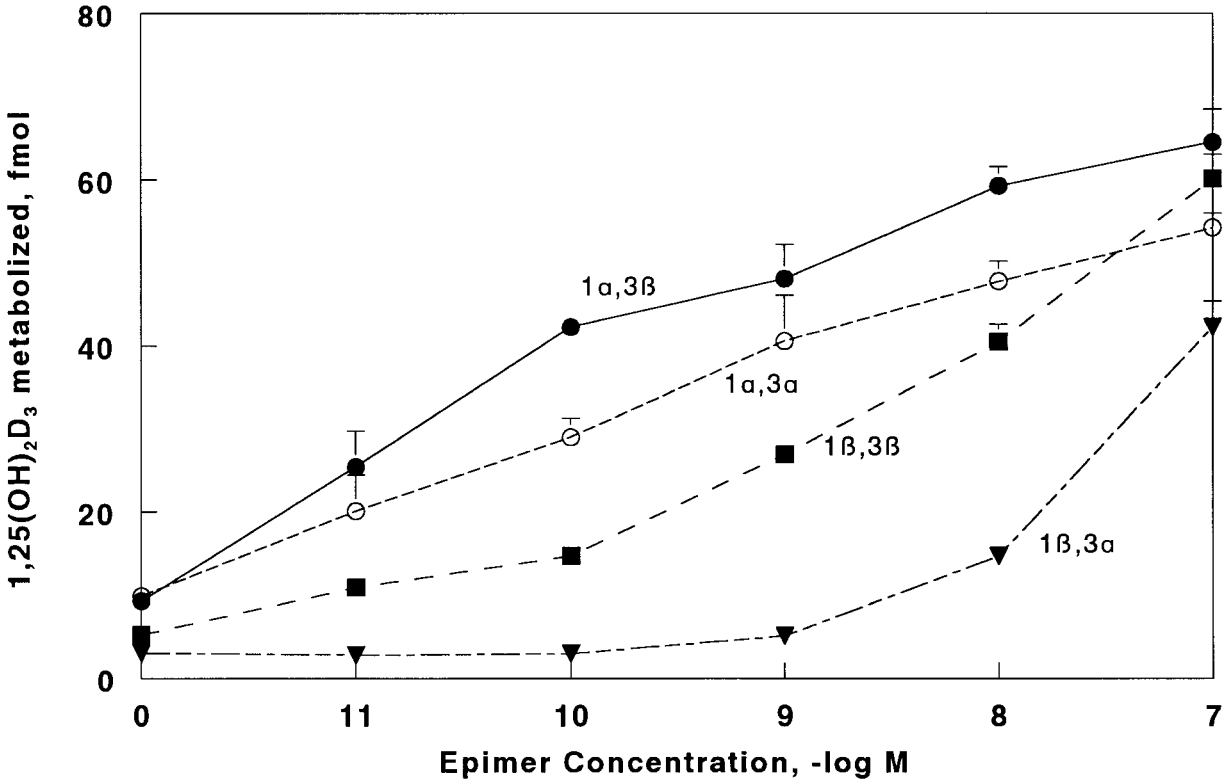

Fig. 3. Induction of 1α,25(OH)$_2$D$_3$ metabolism in parathyroid cells by A-ring diastereomers. Confluent cultures of bovine parathyroid cells were incubated with the specified concentration of 1α,25-(OH)$_2$D$_3$ (1α,3β), 1α,25(OH)$_2$-3-epi-D$_3$ (1α,3α), 1β,25(OH)$_2$D$_3$ (1β,3β), or 1β,25(OH)$_2$-3-epi-D$_3$ (1β,3α) for 24 h. The cells were then incubated with 5 nM [^{3}H]-1α,25-(OH)$_2$D$_3$ for 30 min. The cells and media were extracted and analyzed by HPLC to determine the amount of [^{3}H]-1α,25-(OH)$_2$D$_3$ remaining. Parallel samples with no cells were used as controls. The difference between the control and experimental samples is expressed as the amount of 1α,25(OH)$_2$D$_3$ metabolized (mean ±S.D., n = 3).

VDR affinity of the A-ring diastereomers was determined in intact parathyroid cells (Fig. 4). 1β,25(OH)$_2$D$_3$ appeared to have significant affinity for the VDR, in contrast to reports that this analog does not bind the chick intestinal VDR [Bouillon et al., 1995]. However, 1β,25(OH)$_2$-3-epi- D$_3$ had extremely low affinity for parathyroid cell VDR and did not produce even a 50% reduction in [^{3}H]1α,25(OH)$_2$D$_3$ binding at a 500-fold excess; this agrees well with the binding to chick intestinal VDR [Bouillon et al., 1995].

DISCUSSION

We have previously shown that 1α,25(OH)$_2$D$_3$ metabolism proceeds slowly in cultured bovine parathyroid cells grown in serum-free medium but can be greatly enhanced by pretreatment of the cells with 1α,25(OH)$_2$D$_3$ [Brown et al., 1992]. In the present study we examined in greater detail the fate of 1α,25(OH)$_2$D$_3$ in these cells. HPLC analysis showed several peaks more polar than 1α,25(OH)$_2$D$_3$ with the distinct ultraviolet absorption spectra of vitamin D compounds. These peaks coeluted with authentic standards for the metabolites of the C-24 oxidation pathway: 1α,24(R),25- (OH)$_3$-vitamin D$_3$, 1α,25-(OH)$_2$-24-oxo-vitamin D$_3$, 1α,23(S),25-(OH)$_3$-24-oxo-vitamin D$_3$, and 1α,23-(OH)$_2$-tetranor-vitamin D$_3$. The identity of one of these metabolites, 1α,23(S),25-(OH)$_3$-24- oxo-vitamin D$_3$ was also confirmed by mass spectroscopy and found to be a potent suppressor of PTH secretion [Lee et al., 1997]. The other major metabolic pathway for 1α,25(OH)$_2$D$_3$ observed in vivo and in other cell types produces 1α,25(OH)$_2$D$_3$-26,23-lactone. Under the conditions employed in our experiments, we could not assess the production of the lactone in parathyroid cells.

A major product consistently formed during incubations with parathyroid cells was the 1α,25(OH)$_2$-3-epi-D$_3$. This compound also has been identified recently as a metabolite of 1α,25(OH)$_2$D$_3$ in cultures of keratinocytes and osteoblasts (unpublished data), and in Caco-2 colonic epithelial cells [Bischof et al., 1998]. It

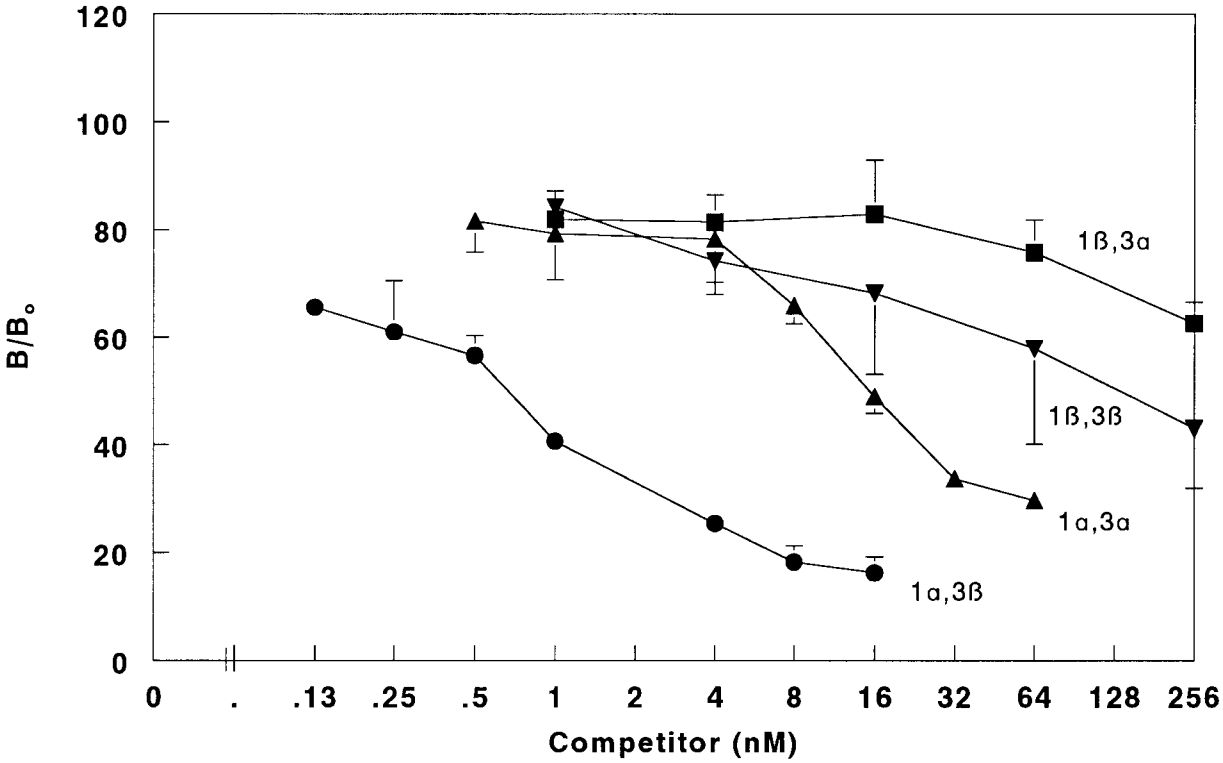

Fig. 4. VDR affinities of A-ring diastereomers in parathyroid cells. Confluent parathyroid cells were incubated for 2 h with 0.5 nM 1α,25-$(OH)_2$-[26,27-^{3}H]D_3 and the indicated concentration of radioinert 1α,25-$(OH)_2D_3$ (1α,3β),1α,25$(OH)_2$-3-epi-D_3 (1α,3α), 1β,25$(OH)_2D_3$ (1β,3β) or 1β,25$(OH)_2$-3-epi-D_3 (1β,3α) in serum-free medium containing 0.5 mg BSA/ml. Binding to the VDR was determined as described under Materials and Methods. Data are mean ±S.D., n = 3.

is of interest that by the end of the incubations of parathyroid cells with 1α,25$(OH)_2D_3$, 1α,25$(OH)_2$-3-epi-D_3 is present at higher levels than the remaining 1α,25$(OH)_2D_3$, and suggests that the 1α,25$(OH)_2$-3-epi-D_3 is metabolized more slowly. This could explain the higher than predicted activity, based on the VDR affinity. VDR affinity was determined during a 2-h incubation, while suppression of PTH secretion was measured after 3 days of treatment with daily changes of medium. We have observed that the parathyroid cell cultures can rapidly metabolize 1α,25$(OH)_2D_3$, completely degrading 5 nM substrate in the medium within 4–6 h. Thus, there may be a slight recovery from the suppression by 1α,25$(OH)_2D_3$, but less recovery from the suppressive effects of the more slowly metabolized 1α,25$(OH)_2$-3-epi-D_3.

Examination of the activities of the other A-ring diastereomers, 1β,25$(OH)_2D_3$ and 1β,25$(OH)_2$-3-epi-D_3 produced some unexpected findings. First, 1β,25$(OH)_2D_3$ had significant affinity for the VDR, although much less than 1α,25$(OH)_2D_3$. Previous studies had reported little if any affinity of 1α,25$(OH)_2D_3$ for the VDR [Bouillon et al., 1995]. We also observed that 1β,25$(OH)_2D_3$ could suppress PTH secretion at high concentrations. The drop in PTH secretion between 10^{-8} M and 10^{-7} M was observed consistently in three independent experiments. It is likely that this suppressive effect on PTH is mediated by interaction of 1β,25$(OH)_2D_3$ with the VDR. However, 1β,25$(OH)_2D_3$ can also interact with a cell surface receptor, but appears to act only as an antagonist, with no apparent agonist activity [Norman et al., 1993]. While it is known that 1α,25$(OH)_2D_3$ can rapidly stimulate phosphoinositide metabolism [Bourdeau et al., 1990] and increase cytosolic calcium [Sugimoto et al., 1988], these rapid effects produce no acute changes in PTH secretion. A role for the rapid responses in transcriptional regulation by 1α,25$(OH)_2D_3$ cannot be excluded.

The ability of low concentrations of 1β,25$(OH)_2$-3-epi-D_3 to *increase* PTH is unprecedented. The extremely low VDR affinity of this compound suggests the possibility that this effect may be mediated by a unique pathway. Furthermore, the absence of any 1α,25$(OH)_2D_3$ in the serum-free medium excludes the possibility that the increased PTH secretion by

1β,25(OH)$_2$-3-epi-D$_3$ is due to antagonism with endogenous 1α,25(OH)$_2$D$_3$. This intriguing finding is currently under investigation.

In summary, we have observed that natural metabolites of 1α,25(OH)$_2$D$_3$ produced by parathyroid cells can potently suppress PTH secretion. We reported the effectiveness of 1α,23(S),25(OH)$_3$-24-oxo-D$_3$ previously [Lee et al., 1997], and now demonstrate that 1α,25(OH)$_2$-3-epi-D$_3$ has nearly the same activity as 1α,25(OH)$_2$D$_3$ in suppressing PTH. Its higher than expected activity (based on VDR affinity) is likely due to its slower metabolism. Accumulation of 1α,25(OH)$_2$- 3-epi-D$_3$ in parathyroid cells may contribute significantly to the overall actions of 1α,25(OH)$_2$D$_3$ in the regulation of PTH synthesis and secretion.

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