Synthesis of [3β-^{3}H]-3-Epivitamin D_3 and Its Metabolism in the Rat†

Sally A. Holick,* Michael F. Holick, Jane E. Frommer, John W. Henley, and Judith A. Lenz

ABSTRACT: 3-Epivitamin D_3, the 3α epimer of vitamin D_3, was synthesized, and its biological activity in the rat was evaluated. It was found to be ~4 times less active on a weight basis than vitamin D_3 with respect to intestinal calcium transport, bone calcium mobilization, and calcification score as determined by the line-test assay. Tritiated 3-epivitamin D_3 was prepared, and its metabolism in the rat was compared with that of vitamin D_3 to investigate the reasons for this diminished activity. 3-Epivitamin D_3 was converted to two polar metabolites, for which the chromatographic properties and the origin of biosynthesis (in the liver and kidney, respectively) correspond to 25-hydroxy-3-epivitamin D_3 and 1α,25-dihydroxy-3-epivitamin D_3. The fact that the concentration of 1α,25-dihydroxy-3-epivitamin D_3 in the intestine is half that of 1α,25-dihydroxyvitamin D_3 may be one explanation for the reduced biological activity of this epimer.

Recent progress in vitamin D metabolism has provided new insights into the pathophysiology of various calcium metabolic disorders (Schnoes & DeLuca, 1976; Haussler & McCain, 1977; Holick & Clark, 1978). These new concepts have prompted a resurgence of interest in the chemical synthesis of vitamin D metabolites and analogues (Holick & DeLuca, 1974; Okamura et al., 1974). Many of these new analogues have been used for examination of structure–function relationships of vitamin D. Alterations in the triene system, the position of the C-19 methylene, and removal of one or two carbons from the side chain were found to significantly decrease biological activity (Holick et al., 1972a, 1975). The 3β-hydroxy group is not required for biological activity, but its absence greatly diminishes the biological potency of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$][1] (Okamura et al., 1975).

To date, only a few vitamin D analogues have been radiolabeled, with subsequent analyses of their metabolic fates. Dihydrotachysterol, a pseudo 1α-hydroxy analogue, is metabolized to 25-hydroxydihydrotachysterol, the latter being responsible for biological activity (Hallick & DeLuca, 1971). 1α-Hydroxyvitamin D_3, a 25-deoxy analogue of 1α,25-$(OH)_2D_3$, was found to be rapidly hydroxylated on C-25, and this metabolism correlated well with the appearance and duration of the biological response seen in the intestine and bone (Holick, M. F., et al., 1976; Holick, S. A., et al., 1976). We chose the 3α epimer of vitamin D_3, first synthesized by Inhoffen in 1960 and found to possess 15% of the biological activity of vitamin D on a weight basis in the chick, as a simple model to examine how a small modification in the molecular structure of vitamin D alters biological activity. We radiolabeled this analogue, examined its metabolism in vivo and in vitro, and correlated its metabolism to the biological response seen in intestinal calcium transport. Our data support the proposal that 3-epivitamin D_3 is hydroxylated first in the liver to 25-hydroxy-3-epivitamin D_3 (25-OH-3-epiD_3) and then in the kidney to 1α,25-dihydroxy-3-epivitamin D_3 [1α,25-$(OH)_2$-3-epiD_3]. An evaluation of the affinity of the intestinal cytosol receptor for 1α,25-$(OH)_2D_3$ compared with that for 1α,25-$(OH)_2$-3-epiD_3 demonstrates a dissociation between biological responsiveness and receptor affinity for this analogue.

Materials and Methods

A Packard Tri-Carb Model 3375 liquid scintillation counter equipped with an automatic external standardization system was used for the radioactive determinations. Samples were collected in plastic vial inserts (14 × 45 mm), dried with a stream of air, and dissolved in 4 mL of Insta-Gel (Packard Instrument Co., Downers Grove, IL). A Beckman DBG recording spectrophotometer was used to record ultraviolet (UV) absorption spectra. An AEI MS-9 mass spectrometer with direct-probe inlet at temperatures of 150–200 °C above ambient temperature was used for mass spectrophotometric determinations, whereas nuclear magnetic resonance (NMR) spectra were obtained with a Varian T-60 NMR spectrometer. Infrared (IR) spectra were recorded with a Perkin-Elmer 237 grating spectrophotometer. High-pressure liquid chromatography (LC) was carried out with a μ-Porasil column (0.4 × 30 cm) on a Waters high-pressure liquid chromatograph equipped with a UV detector (254 nm) and recorder. Periodate oxidation was done according to Holick et al. (1972b).

Compounds. 7-Dehydrocholesteryl acetate was purchased from Steraloids, Inc. (Wilton, NH), and was saponified to 7-dehydrocholesterol, which was obtained in crystalline form (mp 149–150 °C) from methanol. Crystalline vitamin D_3 was obtained from Philips-Roxane, Inc. (NY), and ^{3}H-labeled sodium borohydride (sp act. 5 Ci/mM) was purchased from New England Nuclear (Boston, MA). Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). Melting points were recorded on a Mettler FP2. Silica gel GHLF plates 250 μm thick (Analtech, Inc., Newark, DE) were used for thin-layer chromatography.

Synthesis of 3-Epivitamin D_3. An Oppenauer oxidation (Figure 1) was used to generate 3-ketocholesta-4,7-diene (**2** in Figure 1) from 7-dehydrocholesterol (**1** in Figure 1). To 25 mL of dry refluxing toluene was added **1** (500 mg, 1.30 mmol). When 2 mL of toluene had been collected with a Dean–Stark trap, cyclohexanone (3.00 mL, 3.00 mmol) was added. After an additional 2 mL of toluene had been collected in the trap, 136 mg (0.666 mmol) of aluminum isopropoxide was added rapidly, and the reaction continued to reflux for 30 min. Upon being cooled, the reaction mixture was dis-

† From the Endocrine Unit, Massachusetts General Hospital, and Harvard Medical School, Boston, Massachusetts 02114. *Received January 2, 1980.* This work was supported by Fellowship No. 1 F 32 AM05856-01 and by Grant AM 25506-01 from the National Institute of Arthritis, Metabolism and Digestive Diseases.

[1] Abbreviations used: 25-OH-3-epiD_3, 25-hydroxy-3-epivitamin D_3; 1α,25-$(OH)_2$-3-epiD_3, 1α,25-dihydroxy-3-epivitamin D_3; 25-OHD_3, 25-hydroxyvitamin D_3; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; LC, high-pressure liquid chromatography; IR, infrared spectra; NMR, nuclear magnetic resonance.

0006-2960/80/0419-3933$01.00/0

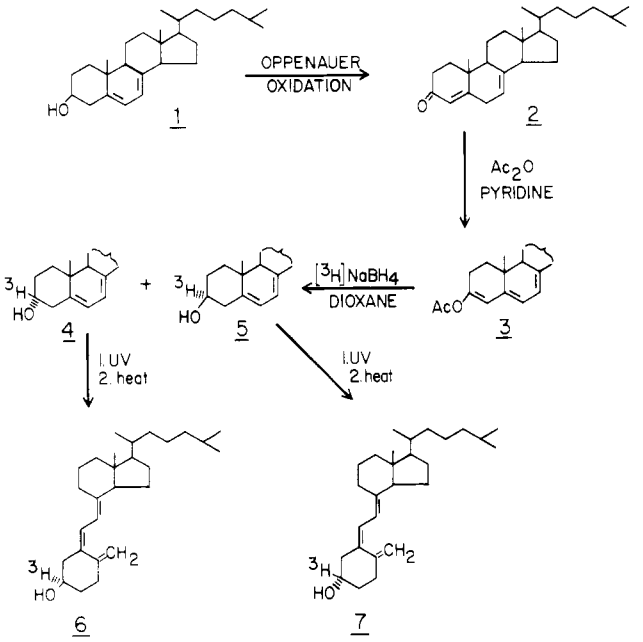

FIGURE 1: Synthetic route for the preparation of [3β-^{3}H]-3-epivitamin D_3 and [3α-^{3}H]vitamin D_3.

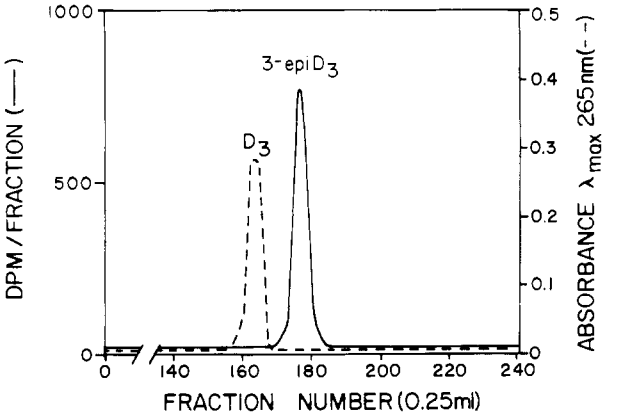

FIGURE 2: High-pressure liquid chromatographic profile of [3β-^{3}H]-3-epivitamin D_3 (3-epiD_3) and crystalline vitamin D_3 (D_3) on a μ-Porasil column (30 cm × 4 mm) developed with 0.25% 2-propanol in *n*-hexane at a pressure of 500 psi and a flow rate of 1 mL/min.

tributed between ether and water, the water layer was withdrawn, and the ether layer was washed twice with 0.1 N hydrochloric acid and then twice with water. The ether layer was filtered through anhydrous sodium sulfate and dried under nitrogen to an oil. A yellow powder of **2** (328 mg, 0.857 mmol, 66% yield), having the UV maximum in methanol at 240 nm, was obtained from methanol.

The acetylation of this dienone produced the corresponding enol acetate. Compound **2** (150 mg, 0.392 mmol) was refluxed with 2 mL of acetic anhydride and 0.5 mL of pyridine for 4 h. Crystals formed upon cooling, and, after recrystallization from ethanol, 3-acetoxycholesta-3,5,7-triene (**3**) was obtained (125 mg, 0.294 mmol, 75% yield). The product melted at 94.5–97 °C and had UV maxima in methanol at 332, 317, and 303 nm; its IR (KBr) showed the carbonyl band at 1810 cm^{-1}; its NMR ($CDCl_3$) indicated the three acetate protons at δ 2.20, two olefinic protons (H-6 and H-7) at δ 5.65, and an olefinic proton (H-4) at δ 5.90. Elemental analysis for $C_{29}H_{45}O_2$ (M_r 425.68) gave 81.60% C and 10.53% H; expected, 81.83% C and 10.65% H.

The enol acetate **3** (59 mg, 0.14 mmol) was then dissolved in 20 mL of dioxane. To this was added sodium borohydride (10 mg, 0.26 mmol), and the reaction mixture was refluxed for 20 h. The reaction mixture was cooled and distributed between ether and pH 3 water; the water layer was withdrawn, and the ether layer was washed with water and then with aqueous saturated sodium chloride. The ether layer was filtered through anhydrous magnesium sulfate and dried under nitrogen to a powder. Purification of the powder on preparative thin-layer chromatographic plates developed with chloroform–ethyl acetate (8:2 v/v) produced 8 mg of 3-epi-7-dehydrocholesterol (UV spectrum λ_{max} 295, 281, and 272 nm) and 40 mg of 7-dehydrocholesterol, identifiable by comparison with authentic material. In a similar fashion, [3β-^{3}H]-3-epi-7-dehydrocholesterol (**4**) and [3α-^{3}H]-7-dehydrocholesterol (**5**) of specific activity 1.2 Ci/mmol were prepared by the reaction of **3** with ^{3}H-labeled sodium borohydride.

The 5,7-dienes were then converted to the respective D vitamins. For example, **4** in ether was irradiated in a quartz immersion well with a Hanovia high-pressure quartz mercury vapor lamp (Model 654A), and the irradiation mixture was chromatographed on a Nedox 15–18–Sephadex LH-20, 95:5 (v/v, *n*-hexane–chloroform), column. [Nedox 15–18–Sephadex LH-20 is an alkoxy C(15)–C(18) derivative of Sephadex LH-20 synthesized as described by Ellingboe et al. (1970).] The fractions containing [3β-^{3}H]-3-epiprevitamin D_3 were combined, dried under nitrogen, dissolved in methanol, and warmed at 60 °C for 2 h to give [3β-^{3}H]-3-epivitamin D_3 (**6**). The product was purified on a Nedox 15–18–Sephadex LH-20, 95:5, column. When chromatographed by LC, it eluted after vitamin D_3 and was homogeneous (Figure 2). The 3-epivitamin D_3 had a characteristic UV spectrum (λ_{max} 265; λ_{min} 228 nm) in methanol and mass spectrum [*m/e* (rel intensity) 384 (M^+, 50), 271 (17), 253 (8), 136 (100), 118 (65)].

Synthesis of 1α,25-Dihydroxy-3-epivitamin D_3. 1α,25-Diacetoxy-3-epi-7-dehydrocholesteryl acetate (kindly supplied by Dr. Uskoković and Dr. Baggiolini of Hoffmann-La Roche Inc.) was saponified to the corresponding free alcohol and then exposed to UV-B irradiation as previously described for the synthesis of 3-epivitamin D_3. 1α,25-Dihydroxy-3-epiprevitamin D_3 was isolated in pure form by LC (5% 2-propanol in *n*-hexane) and thermally converted to 1α,25-$(OH)_2$-3-epiD_3, which was purified by the same LC system. The mass spectrum demonstrated *m/e* (rel intensity) 416 (M^+, 11), 398 (100), 380 (48), 287 (2), 269 (11), 152 (43), 134 (31), and 59 (89), and the UV spectrum showed λ_{max} (methanol) 265 and λ_{min} 228 nm, characteristic for the 5,6-cis-triene system for the D vitamins.

*Metabolism of [3β-^{3}H]-3-Epivitamin D_3 (**6**) and [3α-^{3}H]Vitamin D_3 (**7**).* Male rats obtained from Holtzman (Madison, WI) were maintained for 4 weeks on a diet deficient in vitamin D and normal in calcium and phosphorus content. Groups of two rats each received intravenously, in 50 μL of 95% ethanol, 650 pmol of either **6** or **7** (in Figure 1). After 24 or 48 h, the animals were killed by exsanguination, and their blood, livers, intestines, and kidneys were collected and extracted as described by Bligh & Dyer (1959). The lipid extracts were dissolved in chloroform–*n*-hexane (65:35 v/v), applied to a glass column (1 × 20 cm) containing 5 g of Sephadex LH-20, slurried, and developed in the same solvent (Holick & DeLuca, 1971). Three rats were nephrectomized and then given intravenous injections of 650 pmol of **6** in 50 μL of 95% ethanol. Twenty-four hours later, the animals were killed and bled, and the intestines were removed. Lipid extracts of blood and intestine were prepared and chromatographed as above.

Incubations of Rat Liver Homogenates. The livers were removed from rats maintained on a diet deficient in vitamin

Table I: Response of Intestinal Calcium Transport and Bone Calcium Mobilization to 3-Epivitamin D_3 and Vitamin D_3

dose	^{45}Ca-serosal/ ^{45}Ca-mucosal	serum Ca (mg/100 mL)
50 μL of 95% EtOH	1.7 ± 0.1[a] (6)	4.4 ± 0.1
325 pmol of vitamin D_3	2.9 ± 0.1 (6)	6.9 ± 0.1
650 pmol of vitamin D_3	3.4 ± 0.1 (6)	7.2 ± 0.1
1625 pmol of vitamin D_3	3.7 ± 0.1 (6)	7.5 ± 0.2
325 pmol of 3-epivitamin D_3	1.8 ± 0.1 (6)	4.5 ± 0.1
650 pmol of 3-epivitamin D_3	1.9 ± 0.1 (6)	5.0 ± 0.1
1625 pmol of 3-epivitamin D_3	3.2 ± 0.1 (6)	6.8 ± 0.1
3350 pmol of 3-epivitamin D_3	4.1 ± 0.2 (6)	7.0 ± 0.1

[a] Plus or minus the standard error of the mean. The numbers in parentheses represent the number of rats in each group.

D but adequate in calcium and phosphorus, and 25% (v/v) homogenates were prepared in 0.25 M sucrose by using a Potter-Elvejhem homogenizer. The appropriate cofactors were added to the homogenate (Bhattacharyya & DeLuca, 1974), and 260 pmol of either **6** or **7** in 25 μL of 95% ethanol was added to the appropriate flask. Aliquots of liver homogenates were also heated at 100 °C for 30 min and then handled in a similar fashion. The mixture was incubated at 37 °C for 2 h. The reaction was then terminated by the addition of methanol–chloroform (2:1 v/v) and extracted (Bhattacharyya & DeLuca, 1974). The lipid extracts were chromatographed on a glass column (1 × 20 cm) containing 5 g of Nedox 15–18–Sephadex LH-20 equilibrated and developed with *n*-hexane–chloroform (9:1 v/v).

Line Test. 3-Epivitamin D_3 was analyzed by the line test by the WARF Institute, Inc. (Madison, WI).

Intestinal Calcium Transport Assay. Weanling rats were fed a diet adequate in calcium and phosphorus but deficient in vitamin D for 2 weeks and were then switched to a diet deficient in vitamin D and low in calcium (0.02%) for an additional 2 weeks. Groups of six rats received either 325, 650, 1625, or 3350 pmol of 3-epivitamin D_3 intrajugularly in 50 μL of 95% ethanol. Likewise, groups of six rats received either 325, 650, or 1625 pmol of vitamin D_3. A control group received 95% ethanol only. Twenty-four hours after administration, the animals were decapitated, and their duodena and blood were collected. Intestinal calcium transport activity was measured by the everted gut sac technique (Schachter et al., 1961). Samples (100 μL) from both the inside and outside of the duodenal sac were spotted on filter-paper disks, dried, and placed in 20-mL counting vials containing 10 mL of Insta-Gel.

Bone Calcium Mobilization. The blood from the rats described above was centrifuged, and 0.1 mL of serum was mixed with 1.9 mL of a 0.1% $LaCl_3$ solution. Serum calcium concentration was determined with a Perkin-Elmer atomic absorption spectrophotometer, Model 303 (Holick et al., 1972a).

Results

The calcification response for the animals receiving 3-epivitamin D_3 was 23% (9.2 units/μg) of that expected for vitamin D_3. Similarly, the intestinal calcium transport response, as determined by the everted gut sac technique, and the bone calcium mobilization response showed that 1625 pmol of 3-epivitamin D_3 gave a biological response similar to that seen in animals receiving between 325 and 650 pmol of vitamin D_3 (Table I). An analysis of the dose response of 3-epivitamin D_3 demonstrates that it is about 2.5–4 times less biologically active than vitamin D_3 (Table I).

When equal doses of either [3β-^{3}H]-3-epivitamin D_3 or [3α-^{3}H]vitamin D_3 were administered to rats deficient in vitamin D maintained on a normal calcium and phosphorus diet, both compounds were metabolized similarly after 24 and 48 h. In the blood, intestine, liver, and kidney [3β-^{3}H]-3-epivitamin D_3 was converted to two polar metabolites that, on Sephadex LH-20, migrated in a pattern similar to that of the 25-hydroxy and 1α,25-dihydroxy derivatives of vitamin D_3 (Figure 3A–D). Furthermore, in nephrectomized rats, 3-epivitamin D_3 was not metabolized to peak II (Figure 3E). On LC, the migration of peak II was the same as that of synthetic 1α,25-$(OH)_2$-3-epiD_3 (Figure 4) but was distinct from that of 1α,25-$(OH)_2D_3$ (Figure 4). The elution position of peak II was not affected by periodate oxidation.

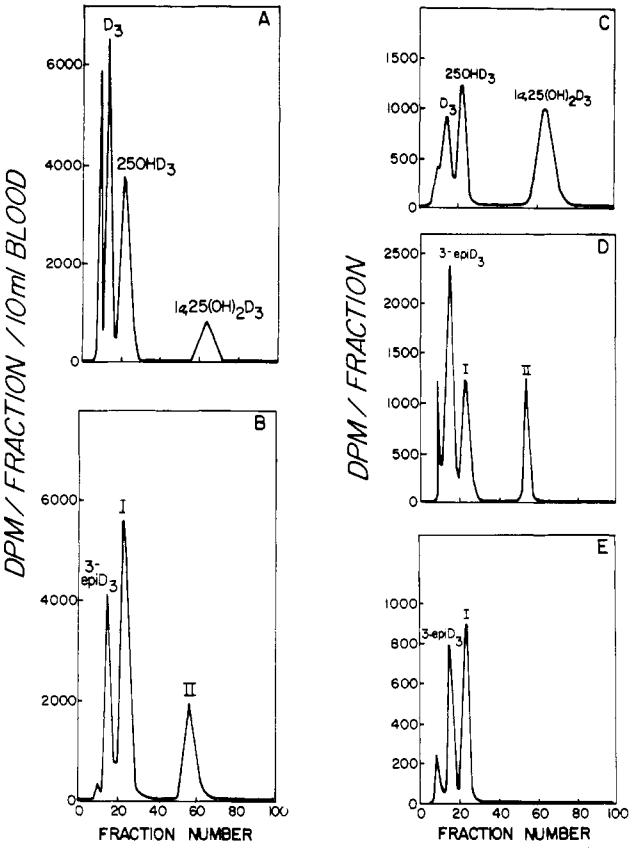

FIGURE 3: Profiles of chromatographed lipid extracts on 5-g Sephadex LH-20 columns [1 × 20 cm, packed and developed with 65:35 (v/v) chloroform–*n*-hexane]. Profile of (A) blood from rats 24 h after receiving 650 pmol of [3α-^{3}H]vitamin D_3, (B) blood from rats 24 h after receiving 650 pmol of [3β-^{3}H]-3-epivitamin D_3, (C) intestine from rats 24 h after receiving 650 pmol of [3α-^{3}H]vitamin D_3, (D) intestine from rats 24 h after receiving 650 pmol of [3β-^{3}H]-3-epivitamin D_3, and (E) intestine from nephrectomized rats 24 h after receiving 650 pmol of [3β-^{3}H]-3-epivitamin D_3.

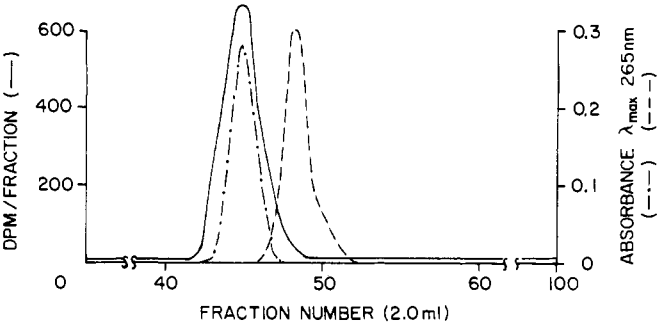

FIGURE 4: High-pressure liquid chromatographic profile of peak II (—), synthetic 1α,25-$(OH)_2$-3-epiD_3 (-·-), and crystalline 1α,25-$(OH)_2D_3$ (- - -) on a μ-Porasil column (30 cm × 4 mm) developed with 5% 2-propanol in *n*-hexane at a pressure of 1000 psi and flow rate of 4 mL/min.

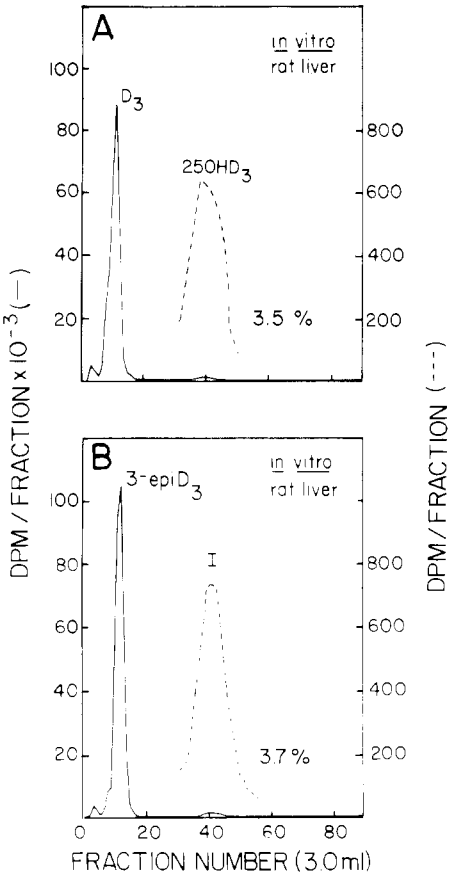

FIGURE 5: Nedox 15–18–Sephadex LH-20 chromatographic profiles of lipid extracts from rat liver homogenates incubated with 260 pmol of either (A) [3α-^{3}H]vitamin D_3 or (B) [3β-^{3}H]-3-epivitamin D_3 for 2 h.

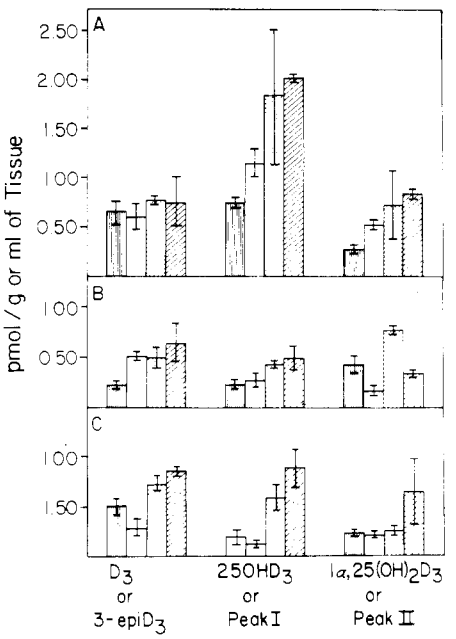

FIGURE 6: Concentrations (pmol/mL or g of tissue) of metabolites in the (A) blood, (B) intestine, and (C) liver 24 and 48 h after dosing with 650 pmol of either [3α-^{3}H]D_3 or [3β-^{3}H]-3-epiD_3. Concentrations of D_3 or its metabolites at 24 h are shown by a vertically striped bar; an open bar indicates the 24-h concentrations of 3-epiD_3 or its metabolites; a dotted bar indicates the 48-h concentrations of D_3 or its metabolites; a hatched bar indicates the 48-h concentrations of 3-epiD_3 or its metabolites. The range is superimposed on each bar.

In rat liver homogenates, [3β-^{3}H]-3-epivitamin D_3 was metabolized to a polar metabolite that migrated in a pattern similar to that of 25-hydroxyvitamin D_3 (25-OHD_3) (Figure 5B). The conversion was ~3.5%, which was identical with the conversion of [3α-^{3}H]vitamin D_3 to 25-OHD_3 by the same homogenates (Figure 5A). Homogenates that were heated to 100 °C for 30 min and incubated with either [3β-^{3}H]-3-epivitamin D_3 or [3α-^{3}H]vitamin D_3 showed 0% conversion.

The concentration of each metabolite in the blood, intestine, liver, and kidney after 24 and 48 h for rats dosed with [3β-^{3}H]-3-epivitamin D_3 and [3α-^{3}H]vitamin D_3 was determined, and some of the results are shown in Figure 6. After 24 h, in the blood, the concentrations of 25-OH-3-epiD_3 and of 1α,25-$(OH)_2$-3-epiD_3 were slightly higher than that of the respective vitamin D_3 metabolites. In the intestine, the concentration of 1α,25-$(OH)_2$-3-epiD_3 was less than half of that of 1α,25-$(OH)_2D_3$ at either 24 or 48 h; the concentrations of 25-OH-3-epiD_3 and 25-OHD_3 did not appear to differ significantly. In the liver and kidney (not shown) there were no significant differences between the concentrations of the vitamin D_3 metabolites and those of the corresponding 3-epivitamin D_3 metabolites. [The 48-h concentration in the liver of 1α,25-$(OH)_2$-3-epiD_3 was not significantly different from the hepatic concentration of 1α,25-$(OH)_2D_3$ at 48 h.]

Discussion

During the past decade, major advances have been made in vitamin D research regarding the isolation, identification, and chemical synthesis of biologically active metabolites. These advances have prompted a vigorous investigation into methods of easily and conveniently synthesizing large quantities of biologically active metabolites and analogues for a wide range of clinical applications. During the past few years, many analogues of vitamin D and its metabolites have been scrutinized. For the most part, these analogues were found to be less active, on a weight basis, than 1α,25-dihydroxyvitamin D_3, and, when compared with the natural hormone, they did not have any unique biological properties.

With the widespread use of 1α-hydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_3 for the treatment of many disorders of calcium metabolism, great interest is focused on the prospect of synthesizing analogues that are true antagonists or strong selective agonists that specifically elicit a biological response in one-organ systems. The design of such effective analogues is dependent on analyses of how the alterations in the structures of various biologically active hormones affect the biological action in target tissue(s).

Here, we report our use of the 3α-hydroxyl epimer of vitamin D_3 as a simple model to carefully analyze why a small alteration in its molecular structure has a dramatic influence on its biological activity. Although 3-epivitamin D_3 does stimulate intestinal transport of calcium, mobilize calcium from bone, and reverse rachitic lesions in rats, it is only about 20–25% as effective as vitamin D_3. Initially, there seemed to be several explanations for the decrease in the biological potency of 3-epivitamin D_3, including the following: (1) defective metabolism of the epimer, i.e., either the vitamin D 25-hydroxylase would not accept 3-epivitamin D_3 as a substrate or it did so inefficiently, or, if 3-epivitamin D_3 was metabolized to 25-hydroxy-3-epivitamin D_3, the latter would not serve as substrate for the renal 25-OHD_3-1α-hydroxylase; (2) rapid degradative metabolism of the epimer; (3) defective target-tissue localization affecting either uptake or binding.

We therefore investigated the metabolism of [3β-^{3}H]-3-epivitamin D_3 in parallel with [3α-^{3}H]vitamin D_3 in rats deficient in vitamin D. Use of a radioactive probe precluded chemical identification of peaks I and II (Figures 3–5), but

multiple criteria indicated that peak I is 25-OH-3-epiD_3 and peak II is 1α,25-$(OH)_2$-3-epiD_3. Analysis of the concentrations of peaks and comparisons with their mono- and dihydroxylated counterparts derived from vitamin D_3 allowed important conclusions concerning differences in metabolism, if any, of the two forms. Chromatographic profiles obtained from the lipid extracts of blood, liver, kidney, and intestine from both groups of animals were remarkably similar at 24 and 48 h after dosing. The metabolite-labeled peak I from the group that received [3β-^{3}H]-3-epivitamin D_3 migrated in a position that is very similar to that of the 25-hydroxy derivative of vitamin D_3. Furthermore, as with 25-hydroxyvitamin D_3, peak I can be made in vitro with liver homogenates from 3-epivitamin D_3. With regard to metabolism by the hepatic 25-hydroxylase, it can be concluded that the percent conversion in vitro is identical with that for the conversion of vitamin D_3 to 25-hydroxyvitamin D_3. The plasma levels of peak I were similar to those of 25-hydroxyvitamin D_3 at equivalent intervals after dosing with the respective precursor, suggesting that the efficiency of the hepatic hydroxylation in vivo is similar for vitamin D_3 and 3-epivitamin D_3.

Similarly, comparison of the lipid-extract chromatograms from rats that received [3β-^{3}H]-3-epivitamin D_3 demonstrates a polar metabolite that migrates very close to, but distinct from, 1α,25-dihydroxyvitamin D_3 in Sephadex LH-20 and LC. The facts that this metabolite (a) is of renal origin, (b) is insensitive to periodate oxidation, and (c) comigrates with synthetic 1α,25-$(OH)_2$-3-epiD_3 strongly support the 1α,25-dihydroxy-3-epivitamin D_3 structure for this metabolite. Thus, hepatic and renal hydroxylations of 3-epivitamin D_3 do not seem to differ from those of vitamin D_3.

With regard to the second possible explanation for the lower biological potency of 3-epivitamin D_3—namely, enhanced metabolic degradation—we determined, at 24 and 48 h after dosing, the tritium content in the feces and urine of rats that had received either [3α-^{3}H]vitamin D_3 or [3β-^{3}H]-3-epivitamin D_3. In each instance, the tritium content was the same for both groups. At 48 h, the urine and fecal tritium content was 5 and 4%, respectively, for rats that received [^{3}H]vitamin D_3 and 4.5 and 3%, respectively, for rats that received [^{3}H]-3-epivitamin D_3. Hence, there appeared to be little, if any, difference in the degradative metabolism of 3-epivitamin D_3.

Results more equivocal to interpret were found with regard to target-tissue localizations. The small intestine showed a marked difference when the concentrations of 3-epivitamin D_3 and its metabolites in the tissue were compared with those for vitamin D_3. The concentration of peak II (which is believed to be the 1α,25-dihydroxy metabolite of 3-epivitamin D_3) in the small intestine is ~50% of that of 1α,25-dihydroxyvitamin D_3. Thus, the decreased potency could be explained in part by the decrease in uptake of the 1α,25-dihydroxy-3-epivitamin D_3 by the target tissue.

Chick intestinal cytosol receptor prepared according to Eisman & DeLuca (1977) showed an affinity for the 3 epimer of 1α,25-$(OH)_2D_3$ that was identical with that for 1α,25-$(OH)_2D_3$ (the native dihydroxy metabolite). These results are somewhat contradictory unless there is a species difference (rats vs. chicks) in the affinity of the intestinal cytosol receptors for this analogue. The similar affinities shown by chick receptors seem to indicate that the reduced uptake of 1α,25-$(OH)_2$-3-epiD_3 in the small intestine does not seem to be related to a problem with the binding of this analogue to the intestinal cytosol receptor. It remains to be determined whether there are other receptors that participate in the process of tissue uptake but are unable to recognize 1α,25-$(OH)_2$-3-epiD_3 as they recognize 1α,25-$(OH)_2D_3$.

With our specific compound, it appears that the hydroxylation of 3-epivitamin D_3 in the liver and kidney is similar to that for vitamin D_3 and that there is not an increase in the metabolic degradation of 3-epivitamin D_3. It seems most likely that the decreased biological activity is linked in some way to the reduced target-tissue concentrations of the 1α,25-derivative of 3-epivitamin D_3. There may be deficiencies in postreceptor induction effects, as well as in the affinity of the presumed receptors for 1α,25-dihydroxy-3-epivitamin D_3. We conclude that systematic examination of the metabolism in vivo and in vitro of analogues of vitamin D may prove a useful approach in determining how changes in the molecular structure affect the biological potency of vitamin D_3. Further studies of the type illustrated here may be useful in dissecting sequential steps in target-tissue response.

Acknowledgments

We very much appreciate the helpful and enthusiastic support of Dr. J. T. Potts, Jr., and the technical assistance of Julia MacLaughlin in the experiments for intestinal calcium transport and bone calcium mobilization and Dr. M. B. Clark for performing the studies for chick intestinal cytosol receptor. We are indebted to the Carbohydrate Research Laboratory for the use of their IR and NMR spectrometers.

References

Bhattacharyya, M., & DeLuca, H. F. (1974) *Arch. Biochem. Biophys. 160*, 58–62.

Bligh, E. G., & Dyer, W. J. (1959) *Can. J. Biochem. Physiol. 37*, 911–915.

Eisman, J. A., & DeLuca, H. F. (1977) *Steroids 30*, 245–257.

Ellingboe, J., Nystrom, E., & Sjovall, J. (1970) *J. Lipid Res. 11*, 266–273.

Hallick, R. B., & DeLuca, H. F. (1971) *J. Biol. Chem. 246*, 5733–5738.

Haussler, M. R., & McCain, T. A. (1977) *N. Engl. J. Med. 297*, 974–983, 1041–1050.

Holick, M. F., & DeLuca, H. F. (1971) *J. Lipid Res. 12*, 460–465.

Holick, M. F., & DeLuca, H. F. (1974) *Adv. Steroid Biochem. Pharmacol. 4*, 115–155.

Holick, M. F., & Clark, M. B. (1978) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 37*, 2567–2574.

Holick, M. F., Garabedian, M., & DeLuca, H. F. (1972a) *Biochemistry 11*, 2715–2719.

Holick, M. F., Schnoes, H. K., DeLuca, H. F., Gray, R. W., Boyle, I. T., & Suda, T. (1972b) *Biochemistry 11*, 4251–4255.

Holick, M. F., Garabedian, M., Schnoes, H. K., & DeLuca, H. F. (1975) *J. Biol. Chem. 250*, 226–230.

Holick, M. F., Tavela, T. E., Holick, S. A., Schnoes, H. K., DeLuca, H. F., & Gallagher, B. M. (1976) *J. Biol. Chem. 251*, 1020–1024.

Holick, S. A., Holick, M. F., Tavela, T., Schnoes, H. K., & DeLuca, H. F. (1976) *J. Biol. Chem. 251*, 1025–1028.

Inhoffen, H. H. (1960) *Angew. Chem. 72*, 875–926.

Okamura, W. H., Norman, A. W., & Wing, R. M. (1974) *Proc. Natl. Acad. Sci. U.S.A. 71*, 4194–4197.

Okamura, W. H., Mitra, M. N., Procsal, D. A., & Norman, A. W. (1975) *Biochem. Biophys. Res. Commun. 65*, 24–29.

Schachter, D., Kimberg, D. V., & Schenker, H. (1961) *Am. J. Physiol. 200*, 1263–1271.

Schnoes, H. K., & DeLuca, H. F. (1976) *Annu. Rev. Biochem. 45*, 631–666.