Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 904-910

INTRINSIC BIOLOGICAL ACTIVITIES BY 1α,24-DIHYDROXYVITAMIN D_3 IN THE RAT

Seiichi ISHIZUKA, Kiyoshi BANNAI, Tatsuyuki NARUCHI, AND Yoshinobu HASHIMOTO

Department of Biochemistry, Teijin Institute for Bio-Medical Research
4-3-2 Asahigaoka, Hino-Shi, Tokyo 191, JAPAN

Received August 10,1979

Summary : Subcellular localization of [^{3}H]1α,24(R)-dihydroxyvitamin D_3 and [^{3}H]1α,24(S)-dihydroxyvitamin D_3 in rat intestinal mucosa was investigated in comparison with the [^{3}H]1α-hydroxyvitamin D_3. The 24(R) and 24(S) isomers of 1α,24-dihydroxyvitamin D_3 were gradually transformed to 1α,24(R)25-trihydroxyvitamin D_3 and 1α,24(S)25-trihydroxyvitamin D_3, and the plasma concentrations of these metabolites were 10.30 and 1.36 pmol/ml, respectively. The major portions of the administered compounds distributed in the nuclear fraction of the intestinal mucosa remained unchanged, and the amounts of 1α,24(R)-dihydroxyvitamin D_3 and 1α,24(S)-dihydroxyvitamin D_3 were 4.25 and 0.306 pmol/g intestinal mucosa, respectively. No detectable amount of the metabolites, 1α,24(R)25-trihydroxyvitamin D_3 and 1α,24(S)25-trihydroxyvitamin D_3 were found in the same nuclear fractions. In the case with the [^{3}H]1α-hydroxyvitamin D_3, however, the compound was rapidly metabolized to 1α,25-dihydroxyvitamin D_3. The metabolite, 1α,25-dihydroxyvitamin D_3, was seen in the nuclear fraction of the intestinal mucosa at a concentration of 2.44 pmol/g intestinal mucosa.

INTRODUCTION

It has been well known that vitamin D_3 exhibits its biological activities in the intestines and bones after undergoing metabolism to 25-OH-D_3 in the liver (1 - 3), and subsequently to 1α,25-$(OH)_2D_3$ in the kidney (4 - 6). The resulting 1α,25-$(OH)_2D_3$ is the active form exerting activities in the intestinal calcium transport (7,8) and the bone calcium mobilization (9) to the extent most potent and to the rate most rapidly among vitamin D_3 analogues.

It has been clarified that the chemically synthesized 1α-OH-D_3 stimulates both the intestinal calcium transport and the bone calcium mobilization as effective as naturally occurring 1α,25-$(OH)_2D_3$ (10 - 13). Further, it was also shown that 1α-OH-D_3 undergoes 25-hydroxylation in the liver (14 - 16) and the resulting metabolite is responsible for the biological activities (17,18)

Abbreviations used : 1α-OH-D_3, 1α-hydroxyvitamin D_3 ; 1α,24(R)-$(OH)_2D_3$, 1α,24(R)-dihydroxyvitamin D_3 ; 1α,24(S)-$(OH)_2D_3$, 1α,24(S)-dihydroxyvitamin D_3 ; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3 ; 1α,24(R)25-$(OH)_3D_3$, 1α,24(R)25-trihydroxyvitamin D_3 ; 1α,24(S)25-$(OH)_3D_3$, 1α,24(S)25-trihydroxyvitamin D_3

0006-291X/79/190904-07$01.00/0

Recently we reported that the chemically synthesized 1α,24(R)-$(OH)_2D_3$ and 1α,24(S)-$(OH)_2D_3$ as well as naturally occurring 1α,25-$(OH)_2D_3$ stimulated the intestinal calcium transport and the bone calcium mobilization quite effectively and rapidly (19,20). The 1α,24-$(OH)_2D_3$ was transformed to 1α,24,25-$(OH)_3D_3$ in the liver, however, it was still unknown whether the biological activities of 1α,24-$(OH)_2D_3$ are due to the parent compound itself or to its metabolite, 1α,24,25-$(OH)_3D_3$. To examine the possibility of whether 1α,24-$(OH)_2D_3$ exerts its biological activity in the target tissue without undergoing metabolism, we determind the subcellular localization of 1α,24-$(OH)_2D_3$ in the intestinal mucosa in comparison with 1α-OH-D_3.

MATERIALS AND METHODS

Materials ——— The [24(S)-^{3}H]1α-OH-D_3, [24-^{3}H]1α,24(R)-$(OH)_2D_3$ and [24-^{3}H]1α, 24(S)-$(OH)_2D_3$ having the specific activities of 3.8, 3.0 and 3.0 Ci/mmol, respectively, were synthesized as described previously (20,21).

Metabolism of 1α,24-$(OH)_2D_3$ and 1α-OH-D_3 ——— Male weanling rats of Wistar strain were fed a vitamin D-deficient, low calcium diet for 6 weeks (20). The vitamin D-deficient rats were given intravenous injections of the [^{3}H]1α,24(R)-$(OH)_2D_3$, [^{3}H]1α,24(S)-$(OH)_2D_3$ or [^{3}H]1α-OH-D_3. The animals were sacrificed 4 hr after the injection and the plasma, liver, kidneys, bones and intestine were isolated. The lipid soluble metabolites were extracted from the plasma, liver, kidneys and bones according to the method described Bligh and dyer (22).

Preparation of Subcellular Fractions of the Small Intestine ——— The small intestine was washed several times with 0.25 M sucrose containing 0.05M Tris-HCl (pH 7.4), 0.025 M KCl and 0.005 M $MgCl_2$ (0.25 M sucrose-TKM). The intestinal mucosa was isolated free of the serosa by scraping with the use of a microscope slide. The intestinal mucosa thus obtained was homogenized with 30 ml of the 0.25 M sucrose-TKM in a Potter - Elvehjem homogenizer equipped with a Teflon pestle. After filtering through two layers of cheesecloth, the homogenate was centrifuged at 800 x g for 10 min. The resulting pellet was designated as the crude nuclear fraction. A fraction containing mitochondria and microsomes was obtained by the further centrifugation of the supernatant fraction at 105,000 x g for 1 hr. The cytosol fraction was also collected.

Column Chromatography ——— The extracts of the tissues of rats treated with [^{3}H]1α,24(R)-$(OH)_2D_3$ or [^{3}H]1α,24(S)-$(OH)_2D_3$ were applied to a Sephadex LH-20 column and eluted with chloroform - n-hexane - methanol (75:23:2). The extracts of the tissues from rats given [^{3}H]1α-OH-D_3 were applied on the same column and eluted with chloroform - n-hexane (65:35).

RESULTS

A 1.0 μg of either [^{3}H]1α,24(R)-$(OH)_2D_3$ or [^{3}H]1α,24(S)-$(OH)_2D_3$ was injected intravenously to vitamin D-deficient rats 4 hr before sacrifice. The extracts of the plasma and the subcellular fractions of the intestinal mucosa were

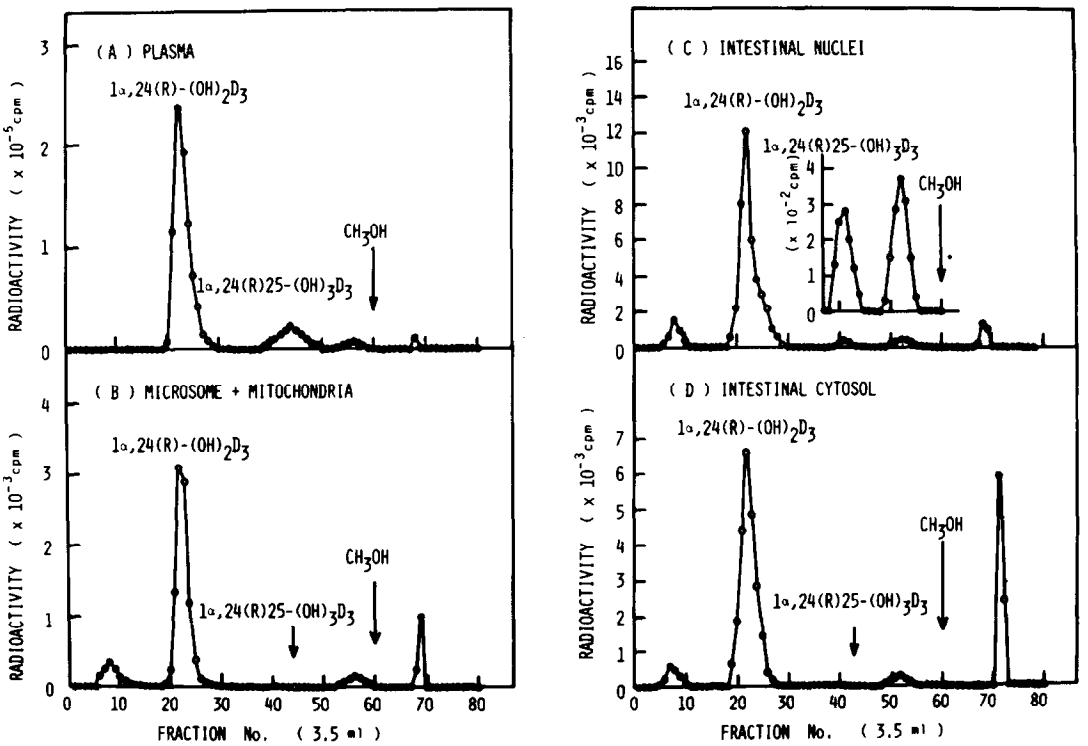

Fig. 1. Elution profiles of the metabolites of 1α,24(R)-$(OH)_2D_3$ extracted from the plasma and the intestine from Sephadex LH-20 column.
See Materials and Methods for details.
(A): plasma (B): intestinal mitochondria plus microsomes
(C): intestinal nuclei (D): intestinal cytosol
The inset in (C) expands the 1α,24(R)25-$(OH)_3D_3$ fraction

applied on the Sephadex LH-20 columns. The elution profiles of the radioactivity are shown in Figs. 1 and 2. Approximately 13 % of the radioactivity extracted to the organic phase from plasma was found to be 1α,24(R)25-$(OH)_3D_3$ (10.30 pmol/ml plasma) as the metabolite of 1α,24(R)-$(OH)_2D_3$. No detectable amount of the metabolite was seen in the nuclear, cytosol or microsomal fraction of the intestinal mucosa, while the unchanged compound was found in larger amounts in these fractions. As was seen with 1α,24(R)-$(OH)_2D_3$, 1α,24(S)25-$(OH)_3D_3$ was present in plasma at a concentration of 1.36 pmol/ml plasma as the metabolite of 1α,24(S)-$(OH)_2D_3$. The amount of 1α,24(S)25-$(OH)_3D_3$ was about 30 % of the total metabolites extracted from plasma. In addition, results similar to those of 1α,24(R)-$(OH)_2D_3$ were also obtained in the distribution pattern of 1α,24(S)-$(OH)_2D_3$.

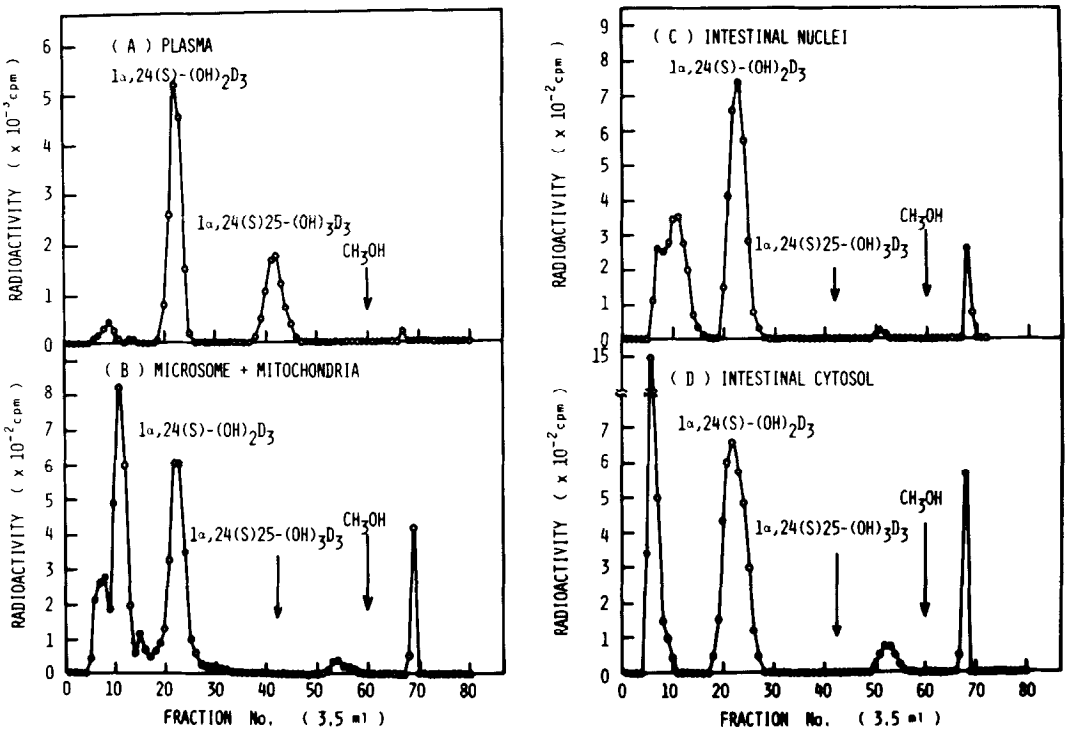

Fig. 2. Elution profiles of the metabolites of 1α,24(S)-$(OH)_2D_3$ extracted from the plasma and the intestine from Sephadex LH-20 column. See Materials and Methods for details.
(A): plasma (B): intestinal mitochondria plus microsomes
(C): intestinal nuclei (D) : intestinal cytosol

Table I summarizes the metabolite levels in the tissues of vitamin D - deficient rats which had received intravenous injection of 1.0 μg of either [^{3}H]1α,24(R)-$(OH)_2D_3$ or [^{3}H]1α,24(S)-$(OH)_2D_3$ 4 hr prior to sacrifice. The unchanged compound was distributed at higher concentrations in the plasma,

Table I. The levels of vitamin D_3 metabolites in the various tissues and the subcellular fractions of the intestinal mucosa.

	1α,24(R)-$(OH)_2D_3$ (pmoles/ml or g tissue)			1α,24(S)-$(OH)_2D_3$ (pmoles/ml or g tissue)			1α-OH-D_3 (pmoles/ml or g tissue)		
	1α,24(R)-$(OH)_2D_3$	1α,24(R)25-$(OH)_3D_3$	Water phase	1α,24(S)-$(OH)_2D_3$	1α,24(S)25-$(OH)_3D_3$	Water phase	1α-OH-D_3	1α,25-$(OH)_2D_3$	Water phase
Plasma	67.50	10.30	15.00	2.92	1.36	12.65	6.70	33.03	15.30
Liver	18.80	1.03	5.30	1.79	0.310	6.42	39.16	12.81	5.37
Bone	2.54	0.163	3.35	0.343	-	1.10	16.42	4.49	0.88
Kidney	10.27	1.15	3.77	0.702	0.091	2.82	14.48	3.22	0.79
Intestinal Mucosa									
Cytosol	2.78	0.013	5.58	0.353	-	1.43	1.02	0.31	3.19
Nuclei	4.25	0.067	1.71	0.306	-	0.18	0.64	2.44	1.42
Mitochondria + Microsome	0.99	-	0.77	0.245	-	0.20	0.61	0.09	0.22

- : undetectable

Vitamin D - deficient rats were given 1 μg of [^{3}H]1α,24(R)-$(OH)_2D_3$, [^{3}H]1α,24(S)-$(OH)_2D_3$ or [^{3}H]1α-OH-D3 intravenously in 0.2 ml of 50 % ethanol. The animals were sacrificed 4 hr after the administration, and various tissues were collected and extracted as described in Materials and Methods.

liver, kidney and the nuclear fraction of the intestinal mucosa, while the 1α,24(R)25-$(OH)_3D_3$ was found mainly in the plasma. Only small amounts of 1α,24(S)-$(OH)_2D_3$ was detected in the plasma and the liver probably due to that 1α,24(S)-$(OH)_2D_3$ was degraded more rapidly than 1α,24(R)-$(OH)_2D_3$.
The amount of 1α,24(S)-$(OH)_2D_3$ in the nuclear fraction of the intestinal mucosa was about one-fourteenth of that seen with 1α,24(R)-$(OH)_2D_3$, which reflected on the intensity of the biological activity (19,20). The 1α-OH-D_3 was assumed to be hydroxylated rapidly to 1α,25-$(OH)_2D_3$ since about 80 % of the total metabolites extracted from the plasma was identified as 1α,25-$(OH)_2D_3$.
On the contrary, large amounts of the unchanged compound were found in the liver, bone and kidney, suggesting that when administered to the rat 1α-OH-D_3 is accumulated in the liver, bone and kidney without undergoing the metabolic transformation. The 1α-OH-D_3 was accumulated especially in the liver, probably indicating that the compound undergoes the 25-hydroxylation. It was also shown that 1α,25-$(OH)_2D_3$ was concentrated in the nuclear fraction of the intestinal mucosa to the level of 2.44 pmol/g tissue.

DISCUSSION

If 1α,25-$(OH)_2D_3$ exerts its biological activity in the chick intestine by a mechanism similar to that seen with steroid hormones (25), then 1α,25-$(OH)_2D_3$ should be accumulated in the nuclear fraction of the intestine.
In other words, it can be expected that 1α,25-$(OH)_2D_3$ binds to cytoplasmic receptor protein first, then transferred to the nucleus to form receptor-chromatin complex (29,30). The resulting complex activates the transcription of the specific genomes that code for functional proteins (31,32).
Thus, it seems reasonable to assume that 1α,25-$(OH)_2D_3$ exerts its action by the same mechanism as seen in the chick. In support of this idea, 1α-OH-D_3 was rapidly metabolized to 1α,25-$(OH)_2D_3$ and it was accumulated in the nuclear fraction of the intestinal mucosa to the concentration of 2.44 pmol/g of intestinal mucosa when administered to vitamin D-deficient rats.
Further, it was also clarified that 1α-OH-D_3 exerts the biological actions

after undergoing metabolism to 1α,25-$(OH)_2D_3$ (Table I). On the contrary to the case of 1α-OH-D_3, 1α,24(R)-$(OH)_2D_3$ and 1α,24(S)-$(OH)_2D_3$ were accumulated in the nuclear fraction in their unchanged forms at the concentrations of 4.25 and 0.306 pmol/g of intestinal mucosa, respectively. The levels of the corresponding hydroxylated products were rather low (Table I).

These results suggest that 1α,24(R)-$(OH)_2D_3$ and 1α,24(S)-$(OH)_2D_3$ exert their biological activities on the intestine without undergoing the 25-hydroxylation reaction. From these evidence, it can be confirmed that among a number of the synthesized vitamin D_3 analogues, 1α,24-$(OH)_2D_3$ is one of the vitamin D_3 analogues which exhibit their biological activities without the further metabolism. This compound will provide a useful tool for clarifying the mechanism of the action of vitamin D_3.

REFERENCES

1. Blunt, J.W., DeLuca, H.F., and Schnoes, H.K. (1968) Biochemistry 7, 3317 - 3322
2. Ponchon, G., and DeLuca, H.F. (1969) J. Clin. Invest. 48, 1273 - 1279
3. Horsting, M., and DeLuca, H.F. (1969) Biochem. Biophys. Res. Commun. 36, 251 - 256
4. Fraser, D.R., and Kodicek, E. (1970) Nature 228, 764 - 766
5. Holick, M.F., Schnoes, H.K., and DeLuca, H.F. (1971) Proc. Natl. Acad. Sci. USA 68, 803 - 804
6. Norman, A.W., Midgett, R.J., Myrtle, J.F., and Nowicki, H.G. (1971) Biochem. Biophys. Res. Commun. 42, 1082 - 1087
7. Boyle, I.T., Miravet, L., Gray, R.W., Holick, M.F., and DeLuca, H.F. (1972) Endocrinology 90, 605 - 608
8. Wong, R.G., Norman, A.W., Reddy, C.R., and Coburn, J.W. (1972) J. Clin. Invest. 51, 1287 - 1291
9. Holick, M.F., Garabedian, M., and DeLuca, H.F. (1972) Science 176, 1146 - 1147
10. Holick, M.F., Semmler, E.J., Schnoes, H.K., and DeLuca, H.F., (1973) Science 180, 190 - 191
11. Haussler, M.R., Zerwekh, J.E., Hesse, R.H., Rizzardo, E., and Pechet, M.M. (1973) Proc. Natl. Acad. Sci. USA 70, 2248 2252
12. Zerwekh, J.E., Brumbaugh, P.F., Haussler, D.H., Cork, D.J., and Haussler, M.R. (1974) Biochemistry 13, 4097 - 4102
13. Holick, M.F., Kasten-Schraufrogel, P., Tavela, T.E., and DeLuca, H.F. (1975) Arch. Biochem. Biophys. 166, 63 -66
14. Fukushima, M., Suzuki, Y., Tohira, Y., Matsunaga, I., Ochi, K., Nagano, H., Nishii, Y., and Suda, T. (1975) Biochem. Biophys. Res. Commun. 66, 632 - 638
15. Fukushima, M., Suzuki, Y., Tohira, Y., Nishii, Y., Suzuki, M., Sasaki, S., and Suda, T. (1976) FEBS Letters 65, 211 - 214
16. Fukushima, M., Nishii, Y., Suzuki, M., and Suda, T. (1978) Biochem. J. 170, 495 - 502
17. Holick, M.F., Tavela, T.E., Holick, S.A., Schnoes, H.K., DeLuca, H.F., and Gallagher, B.M. (1976) J. Biol. Chem. 251, 1020 - 1024

18. Holick, S.A., Holick, M.F., Tavela, T.E., Schnoes, H.K., and DeLuca, H.F. (1976) J. Biol. Chem. 251, 1025 - 1028
19. Kawashima, H., Hoshina, K., Hashimoto, Y., Takeshita, T., Ishimoto, S., Noguchi, T., Ikekawa, N., Morishaki, M., and Orimo, H. (1977) FEBS Letters 76, 177 - 181
20. Ishizuka, S., Bannai, K., Naruchi, T., Hashimoto, Y., Noguchi, T., and Hosoya, N. J. Biochem. (in press)
21. Bannai, K., Ishizuka, S., Naruchi, T., and Hashimoto, Y. (1979) J. Steroid Biochem. 10, 411 - 418
22. Bligh, E.G., and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911 - 917
23. Frolick, C.A., and DeLuca, H.F. (1971) Arch. Biochem. Biophys. 147, 143 - 147
24. Holick, M.F., and DeLuca, H.F. (1971) J. Lipid Res. 12, 460 - 465
25. Buller, R.E., and O'Malley, B.W. (1976) Biochem. Pharmacol. 25, 1 - 12
26. Brumbaugh, P.F., and Haussler, M.R. (1973) Biochem. Biophys. Res. Commun. 51, 74 - 80
27. Tsai, H.C., and Norman, A.W. (1973) J. Biol. Chem. 248, 5967 - 5975
28. Brumbaugh, P.F., and Haussler, M.R. (1974) J. Biol. Chem. 249, 1251 - 1257
29. Brumbaugh, P.F., and Haussler, M.R. (1974) J. Biol. Chem. 249, 1258 - 1262
30. Brumbaugh, P.F., and Haussler, M.R. (1975) J. Biol. Chem. 250, 1588 - 1594
31. Zerwekh, J.E., Haussler, M.R., and Lindell, T.J. (1974) Proc. Natl. Acad. Sci. USA 71, 2337 - 2341
32. Zerwekh, J.E., Lindell, T.J., and Haussler, M.R. (1976) J. Biol. Chem. 251, 2388 - 2394