SIDE CHAIN OXIDATION OF 1,25-DIHYDROXYVITAMIN D_3 IN THE RAT: EFFECT OF REMOVAL OF THE INTESTINE[+]

by

Rajiv Kumar and H. F. DeLuca

Department of Biochemistry

College of Agricultural and Life Sciences

University of Wisconsin-Madison

Madison, Wisconsin 53706

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SUMMARY

Removal of the jejunum, ileum and colon in the rat reduces the amount of $^{14}CO_2$ formed after an intravenous injection of 325 pmoles of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3, by 65.2 $\pm$ 13.2% at 4 hours and 67.1 $\pm$ 9.12% at 8 hours. This suggests that the intestine may be one of the sites where side chain oxidation occurs. It is possible that the liver may also be involved in this process as removal of a large portion of the gut may disturb hepatic metabolism secondary to a reduction in portal blood flow. The process is not bacterial inasmuch as "germ free" animals produce at least as much $^{14}CO_2$ after the administration of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3 as do non-germ free controls.

Evidence has been presented to show that 25-hydroxyvitamin D_3 (25-OH-D_3)[1] and 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) undergo side chain oxidation in the chicken (1) and rat (2, 3). Furthermore, it is likely that only the 1-hydroxylated compounds are immediate precursors in this pathway, inasmuch as nephrectomy and adequate vitamin D levels in the diet abolish or substantially decrease the amount of radiolabeled $^{14}CO_2$ formed after an injection of 25-OH-[26,27-^{14}C]D_3, but not after the administration of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 (2, 3). The functional significance of this pathway is unknown, but the time course of $^{14}CO_2$

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[1]Abbreviations: 25-hydroxyvitamin D_3, 25-OH-D_3; 1,25-dihydroxyvitamin D_3, 1,25-$(OH)_2D_3$.

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formation suggests that it may play some role in the function of 1,25-$(OH)_2D_3$. This investigation was undertaken to establish the site at which the metabolic change occurs. Since the intestine is a target tissue for 1,25-$(OH)_2D_3$ and is easily subjected to surgical removal, the effect of its removal on $^{14}CO_2$ formation after the administration of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 was studied.

MATERIALS AND METHODS

Isotopically Labeled Compounds. 25-OH-[26,27-^{14}C]D_3 was prepared synthetically by the method of Suda et al. (4). 1,25-$(OH)_2$-[26,27-^{14}C]D_3 was prepared enzymatically from 25-OH-[26,27-^{14}C]D_3 using vitamin D-deficient chick kidney mitochondria (5). Both compounds were biologically active, giving antirachitic activity of 180 + 20 units/μg and 410 + 30 units/μg for 25-OH-[26,27-^{14}C]D_3 and 1,25-$(OH)_2$-[26,27-^{14}C]D_3, respectively. Both labeled compounds co-migrated with crystalline 25-OH-D_3 and 1,25-$(OH)_2D_3$ on Sephadex LH-20 (6) and microparticulate silica high-pressure liquid chromatography systems (7), respectively.

Animals. Male, albino weanling rats (Holtzman Co., Madison, Wis.) were housed in overhanging wire cages and fed a vitamin D-deficient, 0.47% calcium, 0.3% phosphorus diet (8) ad libitum for 4 weeks at which time they were used for experimentation.

Five to six week old male, Sprague-Dawley (C-D) rats (Charles River Labs., Charles River, Massachusetts) raised under germ free conditions were obtained. Litter mates raised under normal or non-sterile conditions were used as controls.

To prepare 1,25-$(OH)_2$-[26,27-^{14}C]D_3 from 25-OH-[26,27-^{14}C]D_3, one-day old white leghorn cockerels (Northern Hatcheries, Beaver Dam, Wis.) were fed a rachitogenic diet for 4 weeks (9) at which time they were used to prepare chick kidney mitochondria (5).

Metabolism Apparatus. The rats were placed in a sealed metabolism apparatus (Delmar Scientific Co., Maywood, Illinois) and the carbon dioxide ($^{12}CO_2$ and $^{14}CO_2$) in expired air was trapped in ethanolamine-methyl cellusolve (1:2, v/v) (10). The incoming air was scrubbed free of water by passing it over anhydrous $CaSO_4$ and the CO_2 was removed by passing it over Ascarite (Arthur Thomas Co., Philadelphia, Pa.). The entire apparatus was operated under slight negative pressure achieved by using a water pump vacuum.

The germ free animals were placed in steam sterilized metabolism cages. Incoming air was treated as before except that additional cotton wool air filters were inserted into the air lines. The metabolism cages were placed in an UV irradiation box immediately prior to use in order to remove bacterial contamination. The germ free rats were used immediately upon opening the sterile chamber in which they were transported.

Scintillation Counting. $^{14}CO_2$ trapped in the ethanolamine and methyl cellusolve solution was counted by taking an aliquot of the total volume and counting it in a scintillation solution containing toluene-

Table 1. Effect of Removal of Jejunum, Ileum and Colon on $^{14}CO_2$ Formation After Injection of 1,25-$(OH)_2$-[26,27-^{14}C]D_3

		Group Number		
		1	2	3
Time (hr)	Expt or Control	$^{14}CO_2$ as % dose	$^{14}CO_2$ as % dose	$^{14}CO_2$ as % dose
4	GTX	1.7 ± 0.6*	1.4 ± 0.3*	0.53 ± 0.1++
	Control	3.7 ± 0.7	3.6 ± 0.98	2.6 ± 1.9
8	GTX	6.8 ± 3.1**	3.82 ± 0.4+	
	Control	17.8 ± 1.8	17.1 ± 1.6	--
12	GTX	15.1 ± 6.1++	7.42 ± 0.7+	
	Control	27.0 ± 3.2	26.9 ± 0.8	--

GTX = animals with jejunum, ileum and colon removed. Control and GTX animals received 325 pmoles of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 intravenously immediately prior to being placed in metabolism cages. The values represent the mean ± SD for 3 rats. Statistical analysis was by Student's "t" test.

*p < .02 +p < .001

**p < .01 ++p < .05

methyl cellusolve (2:1, v/v) and 5.5 g of 2,5-diphenyloxazole per liter (10). Radioactive metabolites were counted in a solution containing 2 g of 2,5-diphenyloxazole and 100 mg of 1,4-bis[2-(4-methyl-5-phenoxazolyl)]-benzene per liter of toluene. Radioactivity was determined using a Packard Tricarb scintillation counter (Model 3375) equipped with automatic external standardization.

Anaesthesia. Animals were administered sodium pentobarbital intravenously (30 mg/kg) as an anaesthetic immediately prior to surgery. Control animals also received anaesthetic prior to their sham operation.

Table 2. Percent Decrease in Amount of $^{14}CO_2$ Formation in Animals with Jejunum, Ileum and Colon Removed (GTX)

Time (hr)	Group 1 %	Group 2 %	Group 3 %	Mean ± SD
4	54.1	61.9	79.8	65.2 ± 13.2
8	61.8	77.6		67.1 ± 9.1
12	44.1	72.4		58.2 ± 19.9

The data are calculated from Table 1.

Table 3. $^{14}CO_2$ Formed in Germ Free and Control Rats Dosed with 325 pmoles of $1,25\text{-}(OH)_2\text{-}[26,27\text{-}^{14}C]D_3$ Intravenously

Time (hr)	$^{14}CO_2$ Formed as % Dose Administered Germ free rats	Controls	P value
4	15.7 ± 1.42	8.84 ± 0.7	<.02
8	25.3 ± 1.8	21.7 ± 0.8	<.05
12	29.8 ± 1.7	28.2 ± 0.9	NS
24	33.8 ± 1.04	34.9 ± 1.8	NS

There were 3 animals in each group. The data are expressed as mean ± SD.

RESULTS

Table 1 shows the amount of $^{14}CO_2$ formed in three groups of rats subjected to removal of the jejunum, ileum and colon. Rats in group 3 survived only 6 hours. Thus the results at the 4 hour time point are reported. Rats in the other groups survived 12 hours post operatively.

Each group had three experimental and three control rats. Table 2 shows the percent decrease in $^{14}CO_2$ production when compared with control. Table 3 shows the amount of $^{14}CO_2$ formed in germ free rats and their litter mate controls.

It is clear that removal of the jejunum, ileum and colon substantially decreased by about 60%, the amount of $^{14}CO_2$ formed from 1,25-$(OH)_2$-[26,27-^{14}C]D_3. It is also clear that germ free rats do not form $^{14}CO_2$ in significantly less quantities than their controls. In fact the opposite holds true in that germ animals form more $^{14}CO_2$ at 4 and 8 hours (but not at 12 and 24 hours).

DISCUSSION

It has been established that significant amounts of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 are metabolized to $^{14}CO_2$ by side chain oxidation. Where this process occurs is of obvious possible importance to the function of 1,25-$(OH)_2D_3$ and to further delineating the nature of its metabolism. The results presented above strongly suggest that the intestine plays a role in the side chain oxidation mechanism. A disturbance of hepatic metabolism secondary to disturbance in hepatic portal flow as a result of removal of the jejunum, ileum and colon cannot be excluded, however. It is therefore possible that any one of the following processes occurs:

1) The intestine is involved exclusively in the side chain oxidation of 1,25-$(OH)_2D_3$ with further metabolism of the side chain to CO_2 occurring in the intestine itself. The residual $^{14}CO_2$ formed in the jejuno-ileo-colectomized animals could be formed in the duodenum which is left in place for technical reasons (removal of the duodenum causes damage to pancreas and requires ligation of the bile duct). Alternatively this could be occurring in the other target organ responsive to 1,25-$(OH)_2D_3$, i.e. the bone.

2) The intestine is the organ responsible for side chain cleavage of the 1,25-$(OH)_2D_3$ molecule and the further metabolism of the side

chain occurs in the liver or other organs. Alternatively, all the requisite steps occur both in the intestine and the organ(s) involved.

3) The process is mainly hepatic and the disturbance in portal blood flow is the cause of the observed decrease in $^{14}CO_2$ formation in jejuno-ileo-colectomized animals.

It is also obvious that intestinal bacteria are not responsible for this process to any significant extent as "germ free" animals produce at least as much $^{14}CO_2$ from 1,25-$(OH)_2$-[26,27-^{14}C]D_3 as do non-sterile litter mate controls. Actually, germ free animals form significantly more $^{14}CO_2$ from 1,25-$(OH)_2$-[26,27-^{14}C]D_3 at the 4 and 8 hour time points. The significance of this observation is difficult to interpret; however, it is known that germ free chickens exhibit a greater calcium absorption (11, 12) and germ free rats exhibit hypercalciuria and form renal calculi (12).

Finally, it must be emphasized that experiments of this nature, involving extensive removal of various organs, followed immediately by metabolism measurements must be interpreted with caution. The extensive procedures may cause transitory shock with accompanying metabolic impairment. However, despite these drawbacks it appears likely that the intestine plays an important role in the side chain oxidation pathway for 1,25-$(OH)_2D_3$.

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