1,25-Dihydroxyvitamin D_3 Increases the Activity of the Intestinal Phosphatidylcholine Deacylation-Reacylation Cycle

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ABSTRACT

The activity of the intestinal phosphatidylcholine deacylation-reacylation cycle has been found to be stimulated by 1,25-dihydroxy-vitamin D_3. The stimulation of this cycle thus provides a possible mechanism for the reported retailoring of the fatty acid composition of phosphatidylcholine in intestinal cell membranes by 1,25-dihydroxy-vitamin D_3 and its analogue, 1α-hydroxyvitamin D_3.

INTRODUCTION

Since the demonstration that 1α,25-dihydroxyvitamin D_3 ($1,25(OH)_2$ D_3) is the principal metabolically active form of vitamin D_3 that is involved in calcium homeostasis (1), much interest has been focused on the effects and mechanism(s) of action of this hormonal form of vitamin D and its analogues at the target tissues. It has been demonstrated repeatedly that a major physiological effect of $1,25(OH)_2$ D_3 is to increase the active transport of calcium in the duodenum and upper jejunum of nearly all mammalian and avian species (1). In spite of these demonstrations and studies, the biochemical basis of the action of $1,25(OH)_2$ D_3 is unknown. Walling (2) observed a biphasic response of rat duodenal active calcium absorption following $1,25(OH)_2$ D_3 administration and has suggested that this can be explained by an acute response at 6 hr occurring in the villus cell and a later (24-48 hr) elevation of transport resulting from crypt cells that have subsequently migrated up the villi. Although it has recently been reported (3) that the administration of 1α-hydroxyvitamin D_3, an analogue of $1,25(OH)_2$ D_3, to chicks caused a change in the fatty acid composition of the phosphatidylcholine fraction of the intestinal microvillus membrane, no mechanism was suggested by which this could be achieved.

One established mechanism by which the fatty acid composition of membranes could be retailored is by the phosphoglyceride deacylation-reacylation cycle (4,5). The present report provides the first demonstration that $1,25(OH)_2$ D_3 can stimulate the activities of the enzymes involved in the phosphatidyl choline deacylaton-reacylation cycle in duodenal villus cells of rat intestine.

MATERIALS AND METHODS

Mixed acid 1-acyl-*sn*-1-[1-^{3}H] glycero-3-phosphocholine was prepared as described previously (6) and 1-palmitoyl-2-[1-^{14}C]-oleoyl-*sn*-glycero-3-phosphocholine was prepared according to Lands and Merkl (7). 1,25-Dihydroxyvitamin D_3 was a gift from Dr. M. Uskokovic, Hoffman La-Roche, Nutley, NJ. Weanling male Holtzmann rats were fed the Vitamin D-deficient, low calcium diet of Suda et al. (8) for 3 weeks as previously described (9). They exhibited low serum calcium concentration and averaged 80±10 g of body weight at the time of the experiment. To determine the effect of the vitamin D metabolite on the deacylation-reacylation cycle, the animals received 650 pmol of $1,25(OH)_2$ D_3 intrajugularly in 0.05 ml ethanol. The control group received the vehicle alone. At 3 and 5 hr after dosing, the animals were sacrificed and isolated duodenal villus cells prepared by collagenase dispersion (10). Phospholipase A_2 [E.C. 3.1.1.4] was assayed in duodenal cell homogenates as described by Subbaiah and Ganguly (11), using 6 μmol of 1-palmitoyl-2-[1-^{14}C] oleoyl-*sn*-3-phosphocholine as substrate. Each incubation contained 1.5 mg of cell protein. The reactions were carried out for 60 min at 37 C. Lysophosphatidylcholine acyltransferase [E.C. 2.3.1.23] was assayed in cell homogenates as previously described (12). The incubations were terminated by addition of chloroform-methanol, 2:1, and total lipid extracts were prepared according to the method of Folch et al. (13). The phospholipids were resolved into their various classes by thin layer chromatography using chloroform/methanol/glacial acetic acid/water (25:15:4:4, v/v/v/v) as the developing solvent, and the various lipid fractions were eluted from the silica gel as previously described (6). As radioactive acyl-CoAs were used in the acyltransferase assay, the radioactivity at the *sn*-2 position of the synthesized phosphatidylcholine was determined after phospholipase A_2 hydrolysis (14). Liquid scintillation spectrometry was carried out as described (6). The recovery of the tritium isotope was 92% and the recovery of the ^{14}C isotope was 90%. Protein was determined by

TABLE I

Effect of 1,25(OH)$_2$ D$_3$ on Phospholipase A$_2$ Activity In Rat Intestinal Cells

Experimental condition	Time (hr)	Lysophosphatidyl choline formed[a]	Phosphatidyl choline recovered[a]	Disappearance of total ester bonds[a]
Control	3	252 ± 28	5227 ± 548	228 ± 27
	5	263 ± 27	5348 ± 556	231 ± 24
1,25(OH)$_2$ D$_3$-Treated	3	646 ± 76	4483 ± 463	695 ± 81
	5	921 ± 88	3887 ± 379	993 ± 126

[a]pmol/hr/mg protein. There were five animals in each group. At each time point, the enzyme was assayed in isolated duodenal cells from each animal as described in Materials and Methods using 6 μmol of 1-palmitoyl-2-[1-^{14}C] oleoyl-*sn*-glycero-3-phosphocholine as substrate. Results are the mean ± SEM of five animals.

TABLE II

Lysophosphatidylcholine Acyltransferase Activity In Intestinal Cells from Control and 1,25-Dihydroxyvitamin D$_3$-Treated Rats.[a]

Fatty acyl incubated with 1-acyl-*sn*-[2-^{3}H] glycero-3-phosphocholine	Control		1,25-Dihydroxyvitamin D$_3$-treated	
	3 hr	5 hr	3 hr	5 hr
[1-^{14}C] Oleoyl CoA	882 ± 114	912 ± 102	2238 ± 216	3798 ± 426
[1-^{14}C] Linoleoyl CoA	1098 ± 96	1134 ± 116	3516 ± 384	4764 ± 576
[1-^{14}C] Arachidonyl CoA	1350 ± 144	1278 ± 174	4176 ± 438	5244 ± 546

[a]nmol/hr/mg protein. There were five animals in each group. At each time point, the enzyme was assayed in isolated duodenal cells from each animal as described in Materials and methods. Results are the mean ± SEM of five animals.

the method of Lowry et al. (15) using bovine serum albumin as standard.

RESULTS

The effect of 1,25(OH)$_2$ D$_3$ on phospholipase A$_2$ activity in homogenates of isolated intestinal duodenal cells is shown in Table I. Cell homogenates were chosen for the assay because it has been demonstrated (11) that the enzyme activity is distributed between the brush border, microsomal and cytosolic fractions. The results show that phospholipase A$_2$ activity was increased 2 to 3-fold at 3 hr after administration of 1,25(OH)$_2$ D$_3$, and this was increased to 4-fold at 5 hr. The second enzyme involved in phosphatidylcholine deacylation-reacylation is lysophosphatidylcholine acyltransferase (4,5). Table II shows the effect of 1,25(OH)$_2$ D$_3$ treatment on the activity of this enzyme. In the control experiments, the yield of phosphatidylcholine varied with the acyl CoA used in the assay with arachidonyl CoA being more effective than oleoyl CoA or linoleoyl CoA. 1,25(OH)$_2$ D$_3$ treatment resulted in a 3 to 4-fold stimulation of the enzyme activity without significant alteration of the acyl CoA specificity. These results indicate that 1,25(OH)$_2$ D$_3$ can stimulate the activity of the phosphatidylcholine deacylation-reacylation cycle in intestinal duodenal cells.

DISCUSSION

1,25(OH)$_2$ D$_3$ acts on intestine to stimulate calcium transport (1). Although its structure, biological synthesis (16) and certain features of its mechanism of action (1,17,18) closely resemble other steroid hormones, little is known about the biochemical basis of its mechanism(s) of action in intestinal cells. It had been demonstrated earlier (19,20) that actinomycin D did not prevent the stimulation of intestinal calcium transport by 1,25(OH)$_2$ D$_3$ in rat duodenum. Bikle et al. (21) recently reported similar findings with chick duodenal loops using both actinomycin D and cycloheximide. These authors suggested that the inability of either inhibitor to block 1,25(OH)$_2$ D$_3$-mediated calcium transport despite inhibition of calcium-binding protein production and alkaline phosphatase activity by cycloheximide, indicates that *de novo* RNA and protein synthesis, and, in particular, calcium binding protein and alkaline phosphatase are not required for the 1,25(OH)$_2$ D$_3$ stimulation of calcium transport.

One attractive alternative is a mechanism by which the fatty acid composition of the villus cell membranes could be retailored, thus modifying general properties of the membranes such as fluidity and permeability. This could be affected by increased activity of the phospho-

glyceride deacylation-reacylation cycle (4,5). The results presented above demonstrate that administration of $1,25(OH)_2$ D_3 can increase the activity of the intestinal phospholipase A_2, thus generating lysophosphatidylcholine which can then be reacylated by the acyltransferase reaction, which is also shown to be stimulated by $1,25(OH)_2$ D_3, to form a retailored molecular species of phosphatidylcholine. It is pertinent to point out that we have recently also suggested (22) that the detergent properties of lysophosphatidylcholine may provide the physical basis for a role by this monoacylphospholipid as a metabolic modulator in the intestinal cell.

Yorio and Bentley have recently shown (23) that aldosterone can increase phospholipase A activity in toad bladder and suggested that this was a mechanism by which transepithelial sodium transport was increased. They did not, however, investigate the activity of the acyltransferase reaction. Our present findings provide the first demonstration that $1,25(OH)_2$ D_3 can increase the activities of the enzymes involved in the phosphatidylcholine deacylation-reacylation cycle in intestinal cells. This retailoring of the fatty acid composition of membrane phospholipids by increasing the activity of the deacylation-reacylation cycle could help explain how calcium transport can occur without *de novo* RNA and protein synthesis (21), as well as the change in the fatty acid composition of the phosphatidylcholine fraction of the intestinal microvillus membrane mediated by both 1α-hydroxyvitamin D_3 (3), and $1,25(OH)_2$ D_3 (24). The fact that the enzymes of this cycle can be stimulated 4-fold by 5 hr, could also explain the acute duodenal calcium absorption observed by Walling (2) 6 hr after $1,25(OH)_2$ D_3 administration.

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