PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 177, 455–458 (1984)

# Early and Direct Effect of 1,25-Dihydroxycholecalciferol on Calcium Uptake by Duodena of Rachitic Chicks (41972)

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*Abstract.* Injection of 1,25 dihydroxycholecalciferol ($1,25(OH)_2D_3$, 10 μg) directly into the *in situ* ligated duodenal loop of rachitic chicks significantly elevated the tissue accumulation of $^{47}Ca$ within 20–30 min. The transfer of $^{47}Ca$ from lumen to blood, during the same time period, was not increased nor was there any measurable intestinal calcium-binding protein synthesized. Lesser amounts of $1,25(OH)_2D_3$ (1 or 5 μg) did not result in any statistically significant elevation of $^{47}Ca$ tissue accumulation, nor did they have any effect on $^{47}Ca$ transfer from lumen to blood (transmural). Ten micrograms of $1,24R,25(OH)_3D_3$ was similarly effective in elevating tissue accumulation, whereas $24R,25(OH)_2D_3$ and $25(OH)D_3$ were not. These results provide additional evidence for an early and direct action of $1,25(OH)_2D_3$ in altering intestinal epithelial membrane transport prior to the induction of synthesis of specific transport proteins. 

1,25-Dihydroxycholecalciferol ($1,25(OH)_2D_3$), the most active form of vitamin D in stimulating the intestinal absorption of calcium, induces the synthesis of an intestinal calcium-binding protein (CaBP) (1). The concentration of CaBP correlates well with the degree of calcium absorption under a wide variety of circumstances (2). Recently, a number of reports have appeared suggesting other biological responses to $1,25(OH)_2D_3$ in the intestine, responses that occur rapidly and that apparently cannot be explained by activation of protein synthesis (3–8). Of these, alteration in phospholipid composition of the brush border membrane was suggested as a primary early effect of $1,25(OH)_2D_3$ (7, 8). The lipid composition of cellular membranes has been shown to play an important role in regulating transport processes (9), including transcellular calcium transport.

The present studies demonstrate that $1,25(OH)_2D_3$, when injected into the intestinal lumen of rachitic chicks, affects the accumulation of $^{47}Ca$ by intestinal tissue within 20–30 min. Absorption, i.e., transfer of $^{47}Ca$ from lumen to blood, was unaffected within this time period.

**Materials and Methods.** Chicks used for the $^{47}Ca$ absorption and uptake studies were 3-week-old White Leghorn cockerels, raised after hatching on a rachitogenic (vitamin D-deficient) chick diet (10).

Duodenal absorption was determined by the *in situ* ligated loop procedure, described in detail by Morrissey and Wasserman (11). After the incubation period, the ligated loop was excised and counted intact to determine the percentage absorbed $^{47}Ca$ by difference from the injected dose (100%). Tissue uptake of $^{47}Ca$ was measured by counting the radioactivity in the entire loop after flushing the lumen with 50 ml of cold saline. The metabolites of vitamin $D_3$ were administered intraluminally together with the dosing solution of $^{47}Ca$ (1.0 ml). These were added in ethanol to give a final concentration of 2% ethanol. The same concentration of ethanol was added to the control dosing solution.

Carrier free $^{47}Ca$ (as chloride) in 1 *N* HCl was obtained from Union Carbide Corporation (Oak Ridge, Tenn.). The concentration of $^{47}Ca$ in the various samples was determined by counting in a well-type gamma counter (Model Gamma-300, Beckman Instruments, Inc., Irvine, Calif.) and the counts were expressed as the percentage of the administered dose.

Blood samples were collected by cardiac puncture and the plasma was assayed for

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0037-9727/84 $1.50

calcium and phosphorus. Plasma calcium concentrations were determined by atomic absorption spectrophotometry (Model 360, Perkin–Elmer Co., Norwalk, Conn.), and inorganic phosphorus according to Kraml (12).

For measurement of CaBP, the mucosal tissue was scraped from the duodenum and homogenized (1:4, w/v) in Tris buffer, 0.0137 *M* tris(hydroxymethyl)aminomethane, 0.12 *M* NaCl, pH 7.2. The homogenate was centrifuged at 30,000*g* for 30 min in a refrigerated centrifuge. The CaBP content of the supernatant fluid was determined by the radial immunodiffusion method (13). The supernatant proteins were determined according to the method of Lowry *et al.* (14), using bovine serum albumin as the reference protein.

**Results.** Intraluminal administration of 10 μg of $1,25(OH)_2D_3$ for 30 min resulted in increased uptake of $^{47}Ca$ by the duodena from rachitic chicks (Table I). This apparent increased uptake was not associated with any detectable CaBP nor with changes in $^{47}Ca$ absorption, plasma Ca, or phosphorus concentrations. Lowering the dose of $1,25(OH)_2D_3$ administered intraluminally to 1 or 5 μg increased, on the average, the extent of $^{47}Ca$ uptake by the duodena (Table II), but these were not significantly different from the control value.

To further characterize the early effect of $1,25(OH)_2D_3$ on calcium uptake by the duodena of rachitic chicks, the uptake of $^{47}Ca$ by the ligated segments in response to $1,25(OH)_2D_3$ was studied at various time intervals following the intraluminal administration of the sterol. As seen in Fig. 1, $^{47}Ca$ uptake was increased at 20 and 30 min postinjection when compared with the uptake in duodena of chicks that were not treated with $1,25(OH)_2D_3$ for identical periods of time. Absorption of $^{47}Ca$ increased with time, but the values of the $1,25(OH)_2D_3$ treated group did not differ significantly from the control.

In order to establish whether this early effect of $1,25(OH)_2D_3$ on $^{47}Ca$ uptake by duodena of rachitic chicks is specific for $1,25(OH)_2D_3$, several other metabolites of vitamin $D_3$ were tested. As seen in Table III, $1,25(OH)_2D_3$ and $1,24R,25(OH)_3D_3$ were stimulatory, $24R,25(OH)_2D_3$ showed a small but not statistically significant increase, while $25(OH)D_3$ had no effect.

**Discussion.** The mechanism of action of $1,25(OH)_2D_3$ on stimulating intestinal calcium absorption was considered to be entirely via *de novo* protein synthesis, in accordance with the accepted doctrine of steroid hormone action. More recent investigations have pointed to the multiplicity of action of $1,25(OH)_2D_3$, one on the permeability of the brush border membrane and others on the transfer of $Ca^{2+}$ through and out of the enterocyte (15). The role of CaBP in accelerating the diffusion of $Ca^{2+}$ through the cell interior has been proposed (16, 17), and an effect of vitamin $D_3$ or $1,25(OH)_2D_3$ on the energy-requiring extrusion of $Ca^{2+}$ across the basal-lateral membranes has been reported (18, 19). Part of the evidence for multiple effects of these seco-steroids was that absorption (transfer of $Ca^{2+}$ from intestinal lumen to blood) required sufficient time for protein synthesis to occur, whereas the accumulation of $Ca^{2+}$ by the intestinal tissue occurred much earlier (less than 2 hrs) (8).

The present study demonstrates that $1,25(OH)_2D_3$ can elicit an early and direct effect on $^{47}Ca$ uptake by the duodena of rachitic chicks when administered intralu-

TABLE I. DUODENAL ABSORPTION AND UPTAKE OF $^{47}Ca$ IN RACHITIC CHICKS 30 min FOLLOWING INTRALUMINAL ADMINISTRATION OF 10 μg OF $1,25(OH)_2D_3$

| Diet | Luminal sterol | Absorption (%) | Uptake (%) | Plasma Ca (mg/100 ml) | Plasma phosphorus (mg/100 ml) | CaBP (μg/mg) |
|---|---|---|---|---|---|---|
| Rachitogenic | None | 22.5 ± 2.6[a] | 16.4 ± 2.9[a] | 6.5 ± 0.5[a] | 2.1 ± 0.5[a] | ND |
| Rachitogenic | $1,25(OH)_2D_3$ | 25.8 ± 2.2[a] | 25.7 ± 2.8[b] | 6.2 ± 0.2[a] | 2.3 ± 0.3[a] | ND |

*Note.* Values are means ± SEM of six chicks. ND, not detectable.
[a,b] Means bearing a common superscript within a column are not significantly different ($P \leq 0.05$).

TABLE II. DUODENAL ABSORPTION AND UPTAKE OF $^{47}Ca$ IN RACHITIC CHICKS, 30 min FOLLOWING INTRALUMINAL ADMINISTRATION OF VARIOUS DOSES OF $1,25(OH)_2D_3$

| Luminal $1,25(OH)_2D_3$ ($\mu$g) | Absorption (%) | Uptake (%) |
|---|---|---|
| None | $26.2 \pm 2.3^a$ | $15.8 \pm 1.7^a$ |
| 10 | $31.2 \pm 1.8^a$ | $21.3 \pm 1.6^b$ |
| 5 | $31.1 \pm 1.4^a$ | $18.4 \pm 3.2^a$ |
| 1 | $26.9 \pm 3.5^a$ | $21.1 \pm 3.4^a$ |

*Note.* Values are means ± SEM of six chicks.
$^{a,b}$ Means bearing a common superscript within a column are not significantly different ($P < 0.05$).

TABLE III. DUODENAL ABSORPTION AND UPTAKE OF $^{47}Ca$ IN RACHITIC CHICKS, 20 min FOLLOWING INTRALUMINAL ADMINISTRATION OF 10 $\mu$g OF VITAMIN $D_3$-METABOLITES

| Luminal sterol | Absorption (%) | Uptake (%) |
|---|---|---|
| None | $19.8 \pm 2.95^a$ | $9.5 \pm 1.53^a$ |
| $1,25(OH)_2D_3$ | $22.2 \pm 3.91^a$ | $17.4 \pm 1.54^b$ |
| $1,24R,25(OH)_3D_3$ | $25.8 \pm 2.28^a$ | $16.9 \pm 2.26^b$ |
| $24R,25(OH)_2D_3$ | $21.4 \pm 2.97^a$ | $14.2 \pm 1.98^{ab}$ |
| $25(OH)D_3$ | $17.2 \pm 1.84^a$ | $10.6 \pm 1.00^a$ |

*Note.* Values are means ± SEM of six chicks.
$^{a,b}$ Means bearing a common superscript within a column are not significantly different ($P < 0.05$).

minally for 20–30 min. The 1$\alpha$-hydroxylated derivatives, $1,25(OH)_2D_3$ and $1,24R,25(OH)_3D_3$, were effective whereas $24R,25(OH)_2D_3$ and $25(OH)D_3$ were ineffective under the conditions employed. This points to the specificity of the response. Because of the short time of exposure of the duodenum to the hormone and inability to detect CaBP, it seems that the enhanced $Ca^{2+}$ uptake is not mediated by way of new protein synthesis. Further, the accumulation of $Ca^{2+}$ within the intestinal tissue, not accompanied by a significant increase in absorption, provides evidence for an effect on the permeability properties of the brush border membrane. Although the intraluminal concentrations of vitamin $D_3$ metabolites tested in the present study were high relative to the usual amount injected parenterally (10–300 ng), their effective concentrations were probably quite low when their solubility properties are considered. Certainly, under physiological conditions, the brush border membrane would not be exposed to metabolite levels of this magnitude.

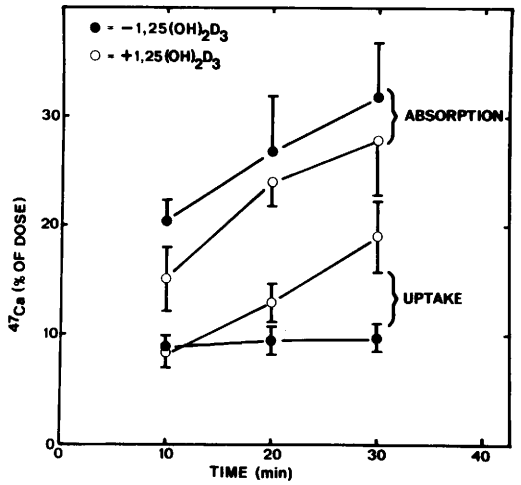


FIG. 1. The effect of intraluminal $1,25(OH)_2D_3$ on $^{47}Ca$ uptake and absorption by the duodena of rachitic chicks as a function of time. Each point represents the mean of six chicks and the error bars, the SEM.

Matsumoto *et al.* (7) were able to demonstrate an effect of parenteral $1,25(OH)_2D_3$ on the $Ca^{2+}$ permeability of isolated brush border membranes within the same time period and Bachelet *et al.* (5) showed that *ex vivo* perfusion of $1,25(OH)_2D_3$ into the vascular system of rat intestine stimulated phosphate ($^{32}P$) absorption and alkaline phosphatase activity by 15 min. Nemere and Norman (20), using an *in vitro* intestinal system, disclosed that $Ca^{2+}$ absorption was increased at 14 min after perfusion of $1,25(OH)_2D_3$ into the vascular system, but *only* in vitamin $D_3$-replete chicks. A rapid effect of $1,25(OH)_2D_3$ on $Ca^{2+}$ accumulation by isolated Golgi membranes was also shown by MacLaughlin *et al.* (6).

In the present study, there was no evidence that *de novo* protein synthesis is required for the action of $1,25(OH)_2D_3$ on $Ca^{2+}$ uptake by the intestinal tissue. Therefore, a direct effect of the hormone on the permeability properties of the brush border membrane is suggested. Other studies, using the same methodology, showed that filipin and A23187, a calcium ionophore, were without effect in amounts up to 100 $\mu$g (unpublished

observations). This further specifies the uniqueness of the activity of the 1$\alpha$-hydroxylated seco-steroids tested in these studies.

This work was supported by NIH Grant AM-04652. The efforts of Francis Davis and Norma Jayne are gratefully acknowledged.

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Received May 5, 1984. P.S.E.B.M. 1984, Vol. 177.
Accepted September 5, 1984.