Ornithine decarboxylase activity in chick duodenum induced by 1 α ,25-dihydroxycholecalciferol

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The effect of cholecalciferol and its metabolites on ornithine decarboxylase activity was investigated in the duodenal mucosa of vitamin D-deficient chicks. The duodenal ornithine decarboxylase activity decreased in animals fed a vitamin D-deficient diet and its retarded activity was increased dose-dependently by a single injection of cholecalciferol. Among various metabolites of cholecalciferol tested, 1 α ,25-dihydroxycholecalciferol [1 α ,25(OH) $_2$ D $_3$] was the most potent stimulator. Stimulation of the enzyme activity was detected as early as 2 h after intravenous administration of 1 α ,25(OH) $_2$ D $_3$ and a maximal value was attained at 6 h. The maximal value was 27 times higher than the control. In addition, treatment with 1 α ,25(OH) $_2$ D $_3$ affected the duodenal content of polyamines. The content of putrescine increased to a value three times that of the control 6 h after the hormone administration. The spermidine content did not change appreciably. The enhancement of duodenal ornithine decarboxylase activity by 1 α ,25(OH) $_2$ D $_3$ occurred in parallel with the enhancement of calcium absorption, which was first detected 3 h after the hormone administration. The enhancement appeared to be tissue-specific. It was observed in every intestinal segment, but was highest in the duodenum. Enzyme activity in other tissues was not influenced appreciably by 1 α ,25(OH) $_2$ D $_3$. These results clearly indicate that the duodenal biosynthesis of polyamines is regulated by 1 α ,25(OH) $_2$ D $_3$, suggesting the possibility that duodenal ornithine decarboxylase may be involved in the calcium absorption mechanism.

Ornithine decarboxylase (EC 4.1.1.17), which catalyses the conversion of ornithine into putrescine, is the rate-limiting enzyme in the biosynthesis of polyamines (Russell & Snyder, 1968; Pegg & Williams-Ashman, 1968). It is known that ornithine decarboxylase activity is markedly enhanced in growing tissues such as regenerating liver (Russell & Snyder, 1968; Hölttä & Jänne, 1972) and developing chick embryos (Russell & Snyder, 1968). In addition, many hormones have been shown to stimulate ornithine decarboxylase activity, resulting in a subsequent increase of polyamine concentrations in their target tissues (Morris & Fillingame, 1974; Maudsley, 1979). Thus polyamines have been considered to be linked to such biological phenomena as cell division, proliferation and differentiation.

1 α ,25(OH) $_2$ D $_3$, the most potent metabolite of cholecalciferol, is now considered to be a hormone that mediates calcium transport in target tissues such as intestine and bone (Kodicek, 1974; DeLuca & Schnoes, 1976). The mechanism of action of 1 α ,25(OH) $_2$ D $_3$ has been extensively examined in chick and rat intestine. It is known that 1 α ,25(OH) $_2$ D $_3$ enters the cell, associates with the specific cytosol receptor and moves into the nucleus (Brumbaugh & Haussler, 1974), where it stimulates RNA polymerase II activity (Zerwekh *et al.*, 1974) and leads to the synthesis *de novo* of specific proteins such as vitamin D-dependent calcium-binding protein (Wasserman *et al.*, 1968; Spencer *et al.*, 1976a). The calcium-binding protein was first identified in the chick intestine by Wasserman *et al.* (1968) and was thought to be the only component of the vitamin D-induced calcium transport system (Spencer *et al.*, 1976a). Subsequently, it has been shown that the kinetic appearance of this protein after administration of 1 α ,25(OH) $_2$ D $_3$ does not precede the

Abbreviations used: 25(OH)D $_3$, 25-hydroxycholecalciferol; 1 α ,25(OH) $_2$ D $_3$, 1 α ,25-dihydroxycholecalciferol; 24,25(OH) $_2$ D $_3$, 24,25-dihydroxycholecalciferol.

enhancement of intestinal calcium absorption (Spencer *et al.*, 1976b), suggesting that there must be some additional factor(s) that is involved in the mechanism of calcium transport. Wilson & Lawson (1977) reported that two of the brush-border membrane proteins are synthesized in response to $1\alpha,25(\text{OH})_2\text{D}_3$ *in vitro* more rapidly than calcium-binding protein. It has also been shown that administration of $1\alpha,25(\text{OH})_2\text{D}_3$ causes an early rise of cyclic GMP concentration in duodenal mucosa (Guillemant & Guillemant, 1980). The relationship between these intestinal proteins or cyclic GMP and the mechanism of the increase in calcium transport remains obscure.

In the present report, we show that administration of $1\alpha,25(\text{OH})_2\text{D}_3$ to vitamin D-deficient chicks significantly increases ornithine decarboxylase activity in chick duodenal mucosa, which occurs no later than the enhancement of intestinal calcium absorption.

Materials and methods

Animals

White Leghorn cockerels (1 day-old) were obtained from Goto poultry farm (Gifu, Japan) and raised on a synthetic vitamin D-deficient diet containing 1.0% calcium and 0.45% phosphorus for 4 weeks (Omdahl *et al.*, 1971). Cholecalciferol and its metabolites were injected intravenously in 0.05 ml of ethanol.

Chemicals

Cholecalciferol, pyridoxal phosphate and dithiothreitol were purchased from Wako Pure Chemicals, Osaka, Japan. L-[1- ^{14}C]Ornithine hydrochloride (sp. radioactivity, 59 Ci/mol) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Crystalline $25(\text{OH})\text{D}_3$ was obtained from Phillips Duphar Co., Amsterdam, The Netherlands. Crystalline $1\alpha,25(\text{OH})_2\text{D}_3$ and $24\text{R},25(\text{OH})_2\text{D}_3$ were kindly donated by Dr. M. R. Uskokovic, Hoffmann-LaRoche Inc., Nutley, NJ, U.S.A. All other chemicals were analytical grades.

Preparation of enzyme solution

The animals were killed by decapitation. Tissues were quickly removed, washed thoroughly with ice-cold 0.9% NaCl, weighed and homogenized with 4 vol. of 25 mM-sodium phosphate buffer containing 5 mM-dithiothreitol and 0.2 mM-pyridoxal phosphate (pH 7.0). The homogenate was centrifuged at 30000g for 30 min and the resulting supernatant was used as an enzyme solution. All procedures were performed at 4°C.

Assay of ornithine decarboxylase activity

Ornithine decarboxylase activity was assayed by a modified method of Russell & Snyder (1968). The

enzyme solution (0.4 ml of 30000g supernatant which contained 8 mg of protein) was transferred into a test tube (16 mm $\times$ 105 mm). The reaction was started by adding 0.1 ml (0.1 μCi) of buffered L-[1- ^{14}C]ornithine hydrochloride. The tube was immediately connected by a 4 cm-long rubber tube to a counting vial to which had been affixed on the inside surface a Whatman 3MM filter paper (3 cm $\times$ 8 cm) soaked in β -phenethylamine. The contents of the tube were incubated for 60 min at 37°C. The incubation medium was then acidified by injecting 0.5 ml of 2M-citric acid through the connecting rubber tube. Incubation continued at 37°C for 1 h to allow the filter paper to absorb evolved $^{14}\text{CO}_2$. The counting vial was then removed and filled with 10 ml of a Triton/toluene scintillation solution containing 12 g of 2,5-diphenyloxazole and 0.6 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 litre of Triton X-100 and 2 litres of toluene. The radioactivity was counted in a Packard liquid-scintillation counter (model 3255).

Assay of intestinal calcium transport activity

Intestinal calcium transport activity was estimated by using the sensitive duodenal-loop-*in-situ* method of Omdahl *et al.* (1971). The duodena of anaesthetized chicks were surgically exposed. After ligating both sides of a 5-cm portion of the duodena, 0.2 ml of phosphate-free [^{45}Ca]calcium bicarbonate solution (120 mM-NaCl, 4.9 mM-KCl, 20 mM- CaCl_2 , 0.5 μCi of ^{45}Ca /ml, 9.2 mM- NaHCO_3 , pH adjusted to 7.0 with CO_2) was injected into the duodenal loop. The duodenal loop was then replaced in the peritoneal cavity. At appropriate times the animals were killed by decapitation and the duodenum was rapidly excised, dried in a porcelain crucible at 100°C for 12 h and subsequently dry-ashed at 600°C for 12 h. The ashed product was dissolved in 2 ml of 2M-HCl. Radioactivity was estimated by dissolving a 100 μl portion of the solution in 10 ml of a dioxan radioactivity-counting solution (100 g of naphthalene and 5 g of 2,5-diphenyloxazole per 1 litre of dioxan) and counting for radioactivity with a Packard liquid-scintillation counter (model 3255). Results, expressed as percentage of ^{45}Ca absorbed, were calculated by the following formula:

$$\% ^{45}\text{Ca}_{\text{abs.}} = 1 - ^{45}\text{Ca}_{\text{R}} / ^{45}\text{Ca}_{\text{A}}$$

where $^{45}\text{Ca}_{\text{R}}$ is the amount of ^{45}Ca remaining in the duodenal loop after the incubation *in situ*, and $^{45}\text{Ca}_{\text{A}}$ is the amount of ^{45}Ca initially added to the loop preparation.

Measurement of polyamine content

Putrescine and spermidine were extracted, isolated and measured by the method of Samejima *et al.* (1976). After intestinal tracts were washed thoroughly with 0.9% NaCl to remove food debris,

tissues were homogenized with 9 vol. of 0.3 M-HClO₄, containing suitable amounts of internal standard, and the homogenate was centrifuged at 3500 rev./min for 15 min at 4°C. A portion of the supernatant was adjusted to pH 5.0 with pyridine and loaded on a CM-cellulose column (5 mm × 50 mm). The column was washed with 0.01 M-pyridine acetate buffer (pH 5.0), and the putrescine was eluted with 3 ml of 0.4 M- and the spermidine with 3 ml of 0.8 M-pyridine acetate buffer (pH 5.0). Each polyamine fraction was collected in a pear-shaped flask and dried *in vacuo* under acetic acid vapour. The residue was solubilized in a minimal volume of 0.01 M-HCl and a portion was transferred to a micro-product vial and dried *in vacuo*. The residue in the vial was solubilized in borate buffer (pH 8.0) and mixed with Fluorescamine (Hoffmann-LaRoche). A portion was loaded on a Hitachi model 638 high-pressure liquid chromatograph. Fluorescence was measured and calibrated by an internal standard. The protein content was estimated by the method of Lowry *et al.* (1951) with bovine serum albumin as standard.

Results

Duodenal ornithine decarboxylase and Ca absorption activities declined linearly when 1-day-old chicks were maintained on a vitamin D-deficient diet. At the end of week 4 of the feeding period, the activities of duodenal ornithine decarboxylase and Ca absorption activities were only one-twentieth and one-third of those in the 1-day-old animals respectively.

Administration of cholecalciferol to the vitamin D-deficient animals enhanced both activities significantly. A marked dose dependence in the stimulation of the duodenal ornithine decarboxylase activity was observed after a single injection of cholecalciferol (Fig. 1a). A maximal stimulation of the enzyme activity was obtained by the dosage of 25 µg of cholecalciferol, and its enhanced activity was 14 times higher than the control. Concomitantly, duodenal Ca absorption activity was increased by the cholecalciferol administration. The maximal stimulation of Ca absorption was obtained by 2.5 µg of cholecalciferol (Fig. 1b).

Fig. 2 shows the time course of change in duodenal ornithine decarboxylase and Ca absorption activities in response to the intravenous injection of 625 ng of 1α,25(OH)₂D₃ into rachitic chicks. A significant stimulation of the duodenal ornithine decarboxylase activity was observed as early as 2 h after 1α,25(OH)₂D₃ injection. The ornithine decarboxylase activity attained a maximal value at 6 h and gradually declined thereafter. At 24 h after injection the ornithine decarboxylase activity declined to the control value (Fig. 2a).

Fig. 1. Dose dependence in the stimulation of duodenal ornithine decarboxylase and calcium absorption activities

Cholecalciferol was administered intravenously to rachitic chicks and the birds were killed 48 h later. Control animals were given ethanol as a vehicle. The assay conditions are described in the Materials and methods section. Duodenal calcium transport activity was measured for 30 min before killing. Results are means ± S.E.M. for five to 19 chicks. (a) Duodenal ornithine decarboxylase activity; (b) duodenal calcium transport activity.

Significant stimulation of duodenal Ca absorption activity by 1α,25(OH)₂D₃ was first detected at 2.5–3 h after injection. It reached a maximum at 12 h and gradually decreased thereafter (Fig. 2b).

Fig. 3 shows the dose-dependence in the stimulation of duodenal ornithine decarboxylase activity 6 h after a single injection of 1α,25(OH)₂D₃. The activity was enhanced significantly by as little as 12.5 ng of 1α,25(OH)₂D₃ and attained a maximum at a dose of 625 ng of 1α,25(OH)₂D₃. Quantitatively 125 ng of 1α,25(OH)₂D₃ was found to be equivalent to 25 µg of cholecalciferol in inducing comparable stimulation of the enzyme activity.

The effect of various metabolites of cholecalciferol on the stimulation of the duodenal ornithine decarboxylase activity is shown in Table 1. In this experiment, ornithine decarboxylase activity was measured 3 h after intravenous injection of each metabolite, because this time period appeared most suitable to detect early stimulation of ornithine

Fig. 2. Time course of change in duodenal ornithine decarboxylase and calcium absorption activities after intravenous administration of 625 ng of 1α,25(OH)₂D₃. Duodenal calcium transport activity was measured for 15 min before killing. Results are means ± S.E.M. for four to 17 chicks. (a) Duodenal ornithine decarboxylase activity (b) duodenal calcium transport activity.

Fig. 3. A dose dependence in the stimulation of duodenal ornithine decarboxylase activity by 1α,25(OH)₂D₃. 1α,25(OH)₂D₃ was intravenously administered to rachitic chicks and the birds were killed 6 h later. Results are means ± S.E.M. for four to 17 chicks.

Table 1. The effect of various metabolites of cholecalciferol on the stimulation of duodenal ornithine decarboxylase activity

Ornithine decarboxylase activity was measured 3 h after intravenous injection of 625 ng of each metabolite. Results are means ± S.E.M., and the values in parentheses are the numbers of birds used.

	Duodenal ornithine decarboxylase activity (pmol of ¹⁴ CO ₂ released/mg of protein)	Ratio of treated/control
Control	4.4 ± 0.5 (19)	
Cholecalciferol	5.8 ± 1.7 (8)	1.3
25(OH)D ₃	8.1 ± 2.9 (10)	1.8
24,25(OH) ₂ D ₃	10.0 ± 3.2 (8)	2.3
1α,25(OH) ₂ D ₃	53.6 ± 8.8 (12)	12.2

decarboxylase activity and to minimize further hydroxylations of the metabolites. Among the various metabolites of cholecalciferol examined, only 1α,25(OH)₂D₃ was found to induce a significant

increase in the duodenal ornithine decarboxylase activity.

Tissue specificity in the enhancement of the ornithine decarboxylase activity induced by 1α,25(OH)₂D₃ is shown in Fig. 4. The ornithine decarboxylase activity in the various tissues was compared 6 h after intravenous injection of 1α,25(OH)₂D₃, since the maximal stimulation of the enzyme activity in the duodenum was obtained at that time. In vitamin D-deficient birds, the ornithine decarboxylase activity in the controls was much higher in the jejunum and ileum than in the duodenum. The ornithine decarboxylase activity in the duodenum, jejunum and ileum were all markedly enhanced by 1α,25(OH)₂D₃, but the ratio of enhanced activities to the control was greatest in the duodenum. Cartilage showed significant ornithine decarboxylase activity in a vitamin D-deficient state, but there was no further enhancement of the enzyme activity by 1α,25(OH)₂D₃. Other tissues showed no significant stimulation of the ornithine decarboxylase activity by 1α,25(OH)₂D₃.

Table 2 shows the time course of change in duodenal concentrations of putrescine and spermidine in rachitic birds after a single injection of 625 ng of 1α,25(OH)₂D₃. The duodenal concen-

Fig. 4. Tissue specificity of the stimulation of ornithine decarboxylase activity by $1\alpha,25(\text{OH})_2\text{D}_3$

The treated birds were given intravenously 625 ng of $1\alpha,25(\text{OH})_2\text{D}_3$. The control birds received vehicle only. All birds were killed 6 h later. The enzyme preparation from the pancreas was carried out in the presence of 0.1 mM-phenylmethanesulphonyl fluoride. Results are means $\pm$ S.E.M..

Table 2. Time course of change in the duodenal contents of putrescine and spermidine in rachitic birds after a single intravenous injection of 625 ng of $1\alpha,25(\text{OH})_2\text{D}_3$

Results are means $\pm$ S.E.M. and the values in parentheses are the numbers of birds used.

Time after $1\alpha,25(\text{OH})_2\text{D}_3$ administration (h)	Duodenal contents (nmol/g of tissue) of:	
	Putrescine	Spermidine
0	95.9 ± 6.3 (5)	954.5 ± 32.8 (5)
3	167.0 ± 8.0 (5)	1105.9 ± 72.2 (5)
6	313.8 ± 22.0 (5)	1181.1 ± 52.4 (5)
12	159.0 ± 4.9 (5)	1110.6 ± 74.6 (5)
24	154.6 ± 12.2 (4)	1181.0 ± 106.6 (4)

tration of putrescine increased significantly at 3 h, attained a maximal value at 6 h and gradually decreased thereafter. These changes were very similar to those in the duodenal ornithine decarboxylase activity induced by $1\alpha,25(\text{OH})_2\text{D}_3$. The content of spermidine, however, remained unchanged throughout the experimental period (Table 2).

Discussion

Many hormones have been shown to stimulate ornithine decarboxylase activity in their respective target tissues. For instance, administration of oestradiol induces ornithine decarboxylase activity

and a subsequent increase in polyamine concentrations in the chick oviduct (Cohen *et al.*, 1970) and rat uterus (Kaye *et al.*, 1971). Somatotropin (Jänne *et al.*, 1968; Russell *et al.*, 1970) and parathyrin (Takigawa *et al.*, 1979) have also been reported to induce ornithine decarboxylase activity in rat liver and rabbit chondrocytes respectively.

In the present study, ornithine decarboxylase activity in the chick duodenum was found to be markedly enhanced by the administration of $1\alpha,25(\text{OH})_2\text{D}_3$ *in vivo*. The K_m value for ornithine has been reported to be about 0.1 mM (Maudsley, 1979). We also found that the K_m value of the chick duodenal ornithine decarboxylase for ornithine was 0.07 mM. Nevertheless, we carried out experiments with limiting concentrations of substrate in the present study, because the ornithine decarboxylase activities in the duodenum and other tissues of vitamin D-deficient chicks were very low. Moreover, when duodenal ornithine decarboxylase activities were measured with 0.5 mM substrate, the activity was increased from 87.8 ± 37.7 ($n = 5$) to 2967.8 ± 321.5 ($n = 5$) pmol of CO_2 released/mg of protein (means $\pm$ S.E.M.) 6 h after injection of 625 ng of $1\alpha,25(\text{OH})_2\text{D}_3$. The ratio of treated value/control value was 33.8, which was similar to that obtained with the limited concentration of substrate. In addition, the duodenal content of putrescine, a polyamine whose synthesis is catalysed by ornithine decarboxylase, increased 3-fold within 6 h in response to $1\alpha,25(\text{OH})_2\text{D}_3$. The induction of duodenal

ornithine decarboxylase activity occurs specifically by $1\alpha,25(\text{OH})_2\text{D}_3$ and appears to be tissue-specific. It is noteworthy that significant stimulation of duodenal ornithine decarboxylase activity occurs as early as 2 h after intravenous injection of $1\alpha,25(\text{OH})_2\text{D}_3$, reaches a maximal value at 6 h and gradually decreases to the control value by 24 h. These quick responses to $1\alpha,25(\text{OH})_2\text{D}_3$ appear to precede the induction of the calcium-binding protein induced by $1\alpha,25(\text{OH})_2\text{D}_3$ (Spencer *et al.*, 1976b).

The current concept of the biochemical action of $1\alpha,25(\text{OH})_2\text{D}_3$ in intestinal tissue is that the hormone enters the cell and stimulates both specific mRNA and protein syntheses (Haussler & McCain, 1977). Wasserman and his colleagues have discovered a calcium-binding protein in chick intestine that is specifically induced by $1\alpha,25(\text{OH})_2\text{D}_3$ (Wasserman *et al.*, 1968). However, the onset of intestinal calcium transport activity in response to $1\alpha,25(\text{OH})_2\text{D}_3$ has been reported to precede the appearance of calcium-binding protein (Spencer *et al.*, 1976b). These results suggest that some unknown $1\alpha,25(\text{OH})_2\text{D}_3$ -dependent factor(s) other than calcium-binding protein is necessary to induce intestinal calcium transport activity.

It has been shown that in response to $1\alpha,25(\text{OH})_2\text{D}_3$ the two brush-border-membrane-bound proteins that have apparent molecular weights of 45 000 and 84 000 are synthesized *in vitro* more rapidly than the calcium-binding protein (Wilson & Lawson, 1977). The syntheses of these two proteins attained maximal values by 3 and 8 h after adding $1\alpha,25(\text{OH})_2\text{D}_3$ to the culture medium respectively, whereas intestinal concentrations of calcium-binding protein attained a maximal value at 21 h (Wilson & Lawson, 1977). Whether ornithine decarboxylase in the duodenum is identical with one of those proteins has to be taken into account in future investigations.

It has been reported that bone (Kream *et al.*, 1977), kidney and pancreas (Christakos & Norman, 1979) also have proteins specifically bound to $1\alpha,25(\text{OH})_2\text{D}_3$ that are similar to a 3.7S cytosol receptor protein found in intestinal tissue. Nevertheless, it is noteworthy that these tissues do not show any significant stimulation of ornithine decarboxylase activity by $1\alpha,25(\text{OH})_2\text{D}_3$.

The polyamine has been shown to be involved in the differentiation and proliferation of various types of cells (Russell & McVicker, 1972). Spielvogel *et al.* (1972) reported that chronic administration of physiological concentrations of cholecalciferol increases the length of the intestinal epithelial villus by 30% in chicks. In the present study the duodenal content of putrescine apparently increased in response to $1\alpha,25(\text{OH})_2\text{D}_3$, suggesting the possi-

bility that one of the possible roles of polyamines in intestinal tissue may be stimulation of the turnover of intestinal epithelial cells. The relationship between polyamines and the duodenal calcium transport mechanism is of considerable interest and requires further study.

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