CONTROL OF CALCIUM ABSORPTION AND INTESTINAL CALCIUM-BINDING PROTEIN SYNTHESIS[1]

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SUMMARY

A current hypothesis suggests that the degree of Ca absorption is hormonally controlled via the feed-back regulation of 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) production from 25-hydroxycholecalciferol (25-OHD_3) by kidney 1-hydroxylase. To test this hypothesis, dihydrotachysterol$_3$ (DHT_3), a steroid not requiring 1-hydroxylation for biological activity, was given to chicks as the only source of vitamin D-activity. As expected, DHT_3-treated chicks did not adapt to a calcium-deficient diet. However, both the efficiency of Ca absorption and net synthesis of CaBP were stimulated in DHT_3-treated chicks by a low phosphorus intake, providing evidence for an alternate pathway of control.

Vitamin D is required for the optimal absorption of calcium in animals and recent evidence indicates that the most effective form of the vitamin ("hormone") is the dihydroxylated derivative, 1,25-dihydroxycholecalciferol (1,25-$(OH)_2D_3$) (1). The hydroxylations occur in two steps, the first via a hydroxylase system in the liver, producing 25-hydroxycholecalciferol (25-$(OH)D_3$ (1) and the second occurs in the kidney, yielding 1,25-$(OH)_2D_3$ (2). Modulation of the formation of 1,25-$(OH)_2D_3$ at the kidney level is thought to be involved in adaptation and in other situations that modify calcium absorption (1). Adaptation in this context refers to the well-

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documented circumstance whereby an animal, initially fed a high calcium diet, increases its efficiency of calcium absorption when fed a low calcium diet (3, 4). Intestinal vitamin D-induced calcium-binding protein (CaBP) concentrations also increase (5-8). Similarly, adaptation occurs on a low phosphate diet which results also in an increase in the efficiency of calcium absorption (6, 7) and an increase in intestinal CaBP levels (6-8). The concentration of CaBP in the intestinal mucosa is highly correlated with the degree of calcium absorption in these and other situations (9).

Factors that have been proposed as being modulators of the kidney hydroxylation reaction, either directly or indirectly, are serum calcium (10), parathyroid hormone (11-14), calcitonin (12, 15), and serum phosphate (16). However, no matter the actual stimulus, the kidney conversion hypothesis denotes that the obligate controllable reaction is hydroxylation of the calciferol steroid nucleus in the 1 position by kidney enzymes. Therefore, it would be unexpected that, if the only source of vitamin D activity were a compound not requiring hydroxylation in the 1 position for biological activity or was an unlikely substrate for the kidney hydroxylase system, no adaptation should occur to either a calcium deficient or phosphorus deficient diet. Such a compound is dihydrotachysterol$_3$ (DHT_3). DHT_3 is a reduction production of vitamin D, with the A ring rotated so that the hydroxyl in the 3 position has a similar geometric position as the 1-hydroxyl of 1,25-$(OH)_2D_3$. DHT_3 is biologically effective in the absence of kidney enzymes, as in nephrectomized animals (17, 18), but is hydroxylated in the liver to form 25-(OH)DHT_3 (19). There is no evidence that hydroxylation of DHT_3 in liver is subject to feed-back inhibition (19).

An evaluation of the ability of animals to adapt to either a low calcium or low phosphorus diet when DHT_3 was their only source of vitamin D activity was undertaken, which is the subject of this report.

MATERIALS AND METHODS

Three separate but identical experiments were carried out.

Day old White Leghorn cockerels were divided into two groups and were fed a vitamin D_3 free but otherwise adequate diet (20). One group was injected intramuscularly every 3 days with 30 IU/chick crystalline vitamin D_3 and the other with 600 IU/chick crystalline DHT_3 in propylene glycol. At the 13th day the chicks from each of the two groups were further divided into three lots and were fed either a control diet (1.05% Ca, 0.75% P), a low calcium diet (0.1% Ca, 0.75% P), or a low phosphorus diet (1.05% Ca, 0.36% P). At day 23, the duodenal absorption of ^{47}Ca was assessed, using the previously described *in situ* ligated loop procedure (21). The duodenal mucosa was then scraped from the underlying tissues, homogenized, centrifuged and heated at 60°C for 10 minutes as previously described (22). After cooling and recentrifugation, the absolute amount of CaBP in the supernatant was determined by a specific radial immunoassay, using highly purified CaBP as the standard (23). Protein concentrations were measured by the Lowry method (24). The treatment means of the 3 experiments were subjected to factorial analysis of variance (diet x experiment) (25).

RESULTS AND DISCUSSION

The results, presented in Table 1, show that duodenal ^{47}Ca absorption and duodenal CaBP concentration were increased significantly ($p<0.001$) in the vitamin D_3-treated chicks fed the low calcium diet as compared to the control group. On the other hand, duodenal ^{47}Ca absorption and duodenal CaBP were no different in the DHT_3-treated chicks on the calcium deficient diet than those on the normal diet, indicating that adaptation did not occur in the former group.

On the low phosphorus diets, duodenal ^{47}Ca absorption and duodenal CaBP concentrations were increased significnatly ($p<0.001$) in chicks given vitamin D_3, as expected (Table 1). Clearly, DHT_3-treated chicks were also capable of partially adapting to the low phosphorus intake (Table 1). Both calcium

Table 1

^{47}Ca Absorption and Intestinal CaBP Levels in Vitamin D_3 or DHT_3-Replete Chicks as a Function of Diet*

	Vitamin D Source			
	D_3		DHT_3	
Diet	^{47}Ca Absorption (% dose)	CaBP (μg/mg protein)	^{47}Ca Absorption (% dose)	CaBP (μg/mg protein)
Control	33.1 ± 2.1	81.6 ± 5.6	30.5 ± 1.4	84.7 ± 3.7
Low Ca	60.9 ± 1.4**	200.0 ± 9.6**	31.8 ± 2.1	83.0 ± 7.7
Low P	49.5 ± 3.0**	189.2 ± 12.6**	39.6 ± 1.7**	126.7 ± 9.3**

* Mean ± S.E. of the individual values taken from three identical experiments. Six chicks on each of the diets in each of 3 experiments.

** Significantly different from respective control group at $p<0.001$.

absorption and CaBP levels were significantly greater than those of the DHT_3-control group. The latter results cannot be due to a vitamin D-contaminant in the DHT_3 preparation; otherwise, the DHT_3-treated low calcium group would have also adapted.

The increase in calcium absorption and duodenal CaBP concentration in the vitamin D_3-replete chicks on the calcium or phosphorus deficient diets agree with previous observations obtained in this laboratory (5, 6) and those from other laboratories (7, 26). Special significance comes from the data obtained with the DHT_3-treated chicks. It is clearly shown (Table 1) that DHT_3-treated chicks cannot adapt to a low calcium intake. Since the physiological action of DHT_3 on intestinal calcium absorption is independent of kidney hydroxylation, those results are consistent with the concept that the regulation of intestinal calcium absorption is mediated by the increased for-

mation of 1,25-$(OH)_2D_3$ by kidney enzymes (1). The appreciable and consistent increase in duodenal ^{47}Ca absorption and CaBP concentration by DHT_3-treated chicks fed the low phosphorus diets (Table 1) was unexpected and not in line with the kidney hydroxylation hypothesis. There should have been no change in calcium absorption and CaBP synthesis if this were the case.

It is too early to speculate on the mechanism of alteration of calcium absorption and CaBP synthesis in the DHT_3-treated, low phosphorus chicks. It is not clear whether the regulation of intestinal calcium absorption by phosphorus restricted chicks is dependent on changes in the production of 25-$(OH)DHT_3$ by the liver, or by a direct interaction between 25-$(OH)DHT_3$ (or DHT_3) and phosphorus at the intestinal level or elsewhere. Whatever the mechanism, this newly uncovered alternate pathway of control could be important in animals on a low phosphorus intake even when the vitamin D source is cholecalciferol. These data also indicate that the mechanism of adaptation occurring on a low calcium diet is not exactly equivalent to that due to a low phosphorus intake.

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