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EARLY RISE IN CYCLIC GMP AFTER 1,25-DIHYDROXYCHOLECALCIFEROL ADMINISTRATION IN THE CHICK INTESTINAL MUCOSA

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SUMMARY

cGMP and cAMP levels were measured in the duodenal mucosa of 12-day-old chicks that had been raised from hatching in vitamin D-depleting conditions and at the time of use were moderately hypocalcemic. After administration of a dose (250 ng) of 1,25-dihydroxycholecalciferol, the cGMP levels increased about twofold in 2-3 hr and returned to control levels between 4 and 6 hr. Our data suggest that 1,25-dihydroxycholecalciferol behaves like other steroid hormones which induce an early rise in cGMP in their respective target tissues.

Various effects of steroid hormones on the concentration of cyclic nucleotides in their respective target tissues have been summarized in a recent review (1). Among them, a striking phenomenon is the estrogen-induced rise in cGMP observed in both the immature rat uterus and the differentiating chick oviduct (2, 3). A similar effect has been described in the rat adrenal cortex after administration of dexamethasone (4).

1,25 $(OH)_2$ D_3 has been demonstrated to be the hormonally active form of vitamin D, and its mechanism of action is like that of other steroid hormones (5). The small intestine mucosa is, together with bone, kidney and parathyroid, one of the target organs for 1,25 $(OH)_2$ D_3. A stimulation of calcium transport and a rise in brush border enzyme activity followed much later by trophic effects on the length of the intestinal villi, are induced by the administration of 1,25 $(OH)_2$ D_3 to vitamin D-deficient animals (6).

Previous reports have focused on a protracted and relatively modest increase of cAMP in the chick or rat small intestine after administration of

ABBREVIATIONS :

1,25 $(OH)_2$ D_3, 1,25-dihydroxycholecalciferol ; 25 OH D, 25-hydroxyvitamin D.

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1,25 $(OH)_2 D_3$ (7, 8). In the present study, we report an earlier rise in cGMP level in the duodenal mucosa of vitamin D-deficient chicks after administration of a physiological dose of 1,25 $(OH)_2 D_3$.

MATERIALS AND METHODS

Animals

One-day-old Warren cockerels were supplied by SFPA, Lorris, France. They were put on a vitamin D-free diet containing 0.8 % Ca and 0.3 % P (C N R Z, La Minière, France) and tap water ad libitum. They were maintained under UV-deprived constant lighting until the age of twelve days.

Experimental procedures

The chicks were killed by cervical dislocation and the blood collected for serum calcium determination. The proximal part (5 cm) of the duodenal loop was excised and washed with chilled saline. The mucosa was then quickly scraped and frozen in dry ice. Next, it was lyophilized and weighed. A solution of 1,25 $(OH)_2 D_3$ (kindly donated by Dr M. Uskokovic, Roche Laboratories, Nutley, New Jersey, U S A) in 50 % saline/ethanol was administered by im injection in the thigh.

Assay for cyclic nucleotides

The lyophilized mucosa was homogenized by sonication in cold N $HClO_4$. After centrifugation the supernatant was alcalinized with 9 M KOH. The precipitate of $KClO_4$ was separated by centrifugation and the supernatant used for cyclic nucleotide assay. Both cyclic nucleotides were determined by radioimmunoassay (9, 10). The excellent specificity of the antibodies used (cross-reactivity between succinyl cAMP and succinyl cAMP and succinyl cGMP less than 0.01 %) allowed direct measurement of the cyclic nucleotides without any previous separation.

Biochemical determination

The pellets obtained after $HClO_4$ precipitation were dried,dissolved in 0.1 N NaOH and diluted for protein determination (11).
Serum calcium was determined by using Corning Calcium Analyzer model 940.
Serum 25-OH D levels were determined by radiocompetitive protein binding assay (12).

Statistical analysis

The values from hormone-treated chicks were analyzed statistically by Student's t-test for paired data versus control animals.

RESULTS

cGMP and cAMP levels were measured in the duodenal mucosa before and at different times after 1,25 $(OH)_2 D_3$ administration. The results of two distinct experiments are presented in Table 1. The base values were around 0.8

TABLE 1 : CYCLIC NUCLEOTIDE LEVELS IN THE DUODENAL MUCOSA OF VITAMIN D-DEFICIENT CHICKS. EFFECT OF 1,25 $(OH)_2 D_3$ ADMINISTRATION.

EXPERIMENT 1	Time after injection hours	n	cGMP % of control mean ± S E M	cAMP % of control mean ± S E M
Untreated control	-	8	100 ± 10	100 ± 9
1,25 $(OH)_2 D_3$[a]	1	8	78 ± 11	120 ± 7
	2	7	208 ± 39[c]	117 ± 11
	4	7	128 ± 12	101 ± 10
	6	7	55 ± 9	143 ± 10[d]
EXPERIMENT 2				
Untreated control	-	14	100 ± 9	100 ± 6
Vehicle control	3	6	89 ± 13	107 ± 9
1,25 $(OH)_2 D_3$[a]	1	8	132 ± 25	113 ± 11
	2	8	136 ± 28	121 ± 6
	2 1/2	8	165 ± 19[d]	116 ± 4
	3	7	169 ± 23[d]	111 ± 9
	4	8	157 ± 31[b]	120 ± 8

a 1,25 $(OH)_2 D_3$ in 50 % ethanol-saline (250 ng/chick) was injected intramuscularly at zero time.

b $p < 0.05$ for difference between this group value and the corresponding control value.

c $p < 0.02$ for difference between this group value and the corresponding control value.

d $p < 0.01$ for difference between this group value and the corresponding control value.

pmole/mg protein for cGMP and 10 pmole/mg protein for cAMP. A significant increase in cGMP was observed at 2 hr during experiment 1, and at 2 1/2 hr, 3 hr and 4 hr during experiment 2. In both experiments the onset of cGMP increase was delayed by about 1-2 hr. The cAMP levels remained at the control values except for a significant increase at 6 hr during experiment 1. Figure 1 shows the intestinal cGMP and serum calcium which were measured during experiment 2. Serum calcium, the base levels of which appear moderately low

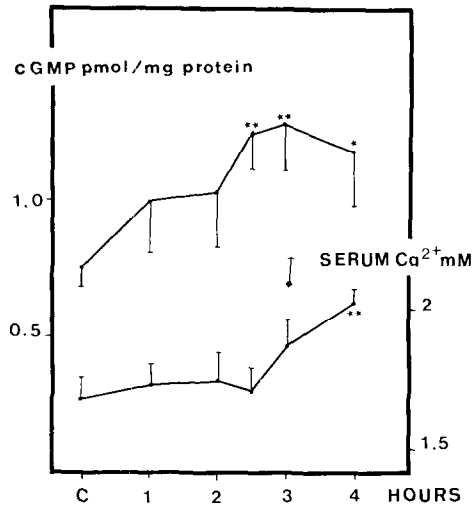

Figure 1. Time course of changes in levels of duodenal cGMP and serum calcium in vitamin D-deficient 12-day-old chicks. cGMP and calcemia were measured at various time intervals after a single im injection of 1,25 $(OH)_2 D_3$ (250 ng in 50 % ethanol-saline/chick). The variation indicated is S E M. ◆ cGMP level in vehicle-injected controls. For the statistical significance of the results, see Table 1 experiment 2.

(mean 6.76 mg/100 ml i.e. 1.69 mM) was significantly increased at 4 hr. As shown in Figure 1, injection of the vehicle alone did not modify the cGMP levels. The 25-OH D serum levels, concurrently determined to assess the effectiveness of vitamin D depletion, were undetectable (< 1 ng/ml).

DISCUSSION

The existence of an effect of 1,25 $(OH)_2 D_3$ on the cellular concentrations of cAMP in the small intestine was investigated some years ago. In vivo studies had indicated a modest elevation of cAMP (around 150 % of the control values) which was manifest at 6 hr and persisted for 24-48 hr (8). Our results (see Table experiment 1), showing an increase at 6 hr, are in agreement with that data. The present findings are related to previous works dealing with the effects of estrogens on cyclic nucleotide levels in the uterus : some authors (2, 13) reported a consistent and significant increase in rat uterine cGMP following estrogen administration. This observation was confirmed by using the chick oviduct (3). On the same token, an elevation in cGMP

has been shown to occur in the rat adrenal cortex after dexamethasone administration (4).

The rise in cGMP that we report in the chick intestine exhibited a similar pattern with a delayed onset, a peak reached between 2-3 hr after injection and a return to control levels at 6 hr. Meanwhile, the cAMP levels were not significantly altered except for the late increase at 6 hr. The modest but significant decrease in cAMP previously found in both the uterus and the adrenal cortex was not seen in this case.

The mechanism as well as the significance of the uterine cGMP increase are only beginning to be elucidated : a time relationship between cGMP rise and uterine wet weight increase has been hypothesized (13). Furthermore, a dependency of the estrogen-induced increase in uterine cGMP upon the estrogen cytosol-nuclear receptor system has been proposed, since a depletion in free cytosol receptors suppresses that increase (14). Although it would be premature to draw definite conclusions about the 1,25 $(OH)_2$ D_3-induced intestinal cGMP increase, we can observe however, that the cGMP response is an early event as compared to a) the stimulation of intestinal calcium transport which culminates at 8-10 hr (15), b) the accumulation of CaBP which is maximal at 24-48 hr (15) or c) the trophic effects which are seen later (6). On the other hand, it should be recalled that the saturation of the intestinal chromatin receptor with injected tritiated 1,25 $(OH)_2$ D_3 is obtained at 3 hr, that is, at the same time as the cGMP response suggesting the possible role of the 1,25 $(OH)_2$ D_3-receptor complex in the induction of the cGMP increase (5).

Components of the cGMP system, guanylate cyclase (16) and cGMP phosphodiesterase (17) are present in the brush border membrane of intestinal cells. Immunohistochemical studies have shown bound cGMP localized at the brush border membrane and in the nuclei of villous cells (18). These data evoke possible sites of action for the 1,25 $(OH)_2$ D_3-induced cGMP accumulation in the chick intestinal cells.

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