STUDIES ON THE MODE OF ACTION OF CALCIFEROL VI: EFFECT OF 1,25-DIHYDROXY-VITAMIN D_3 ON RNA SYNTHESIS IN THE INTESTINAL MUCOSA*

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SUMMARY

Vitamin D (calciferol) has been postulated [A.W. Norman, Science 149, 184 (1965)] to mediate its physiological effects via an inductive process which produces new RNA and proteins. This was tested directly by studying the effect of the biologically active form of calciferol, 1,25-dihydroxycholecalciferol to stimulate in vivo the pulse labeling of intestinal mucosa by ^3H uridine. The maximum stimulation of RNA labeling occurs 6 hours after the intracardial administration of 325 pmoles of 1,25-dihydroxy-cholecalciferol: the first effects were apparent within 3-4 hours. Also, actinomycin D was shown to block both 1,25-dihydroxy-cholecalciferol stimulated Ca^{2+} transport and RNA synthesis. The chronology of events occurring after administration of 1,25-dihydroxy-cholecalciferol suggest that the primary biochemical response of intestinal mucosa to 1,25-dihydroxy-cholecalciferol is probably the initiation of RNA and protein synthesis.

One of the characteristic features of the physiological expression of cholecalciferol[1] activity has been the time lag of 24-36 hours between the administration of CC and the enhancement of calcium absorption across the intestinal mucosa (1). Recent evidence has shown that CC must undergo at least two metabolic conversions prior to the mediation of its biological responses First CC is converted to its 25-hydroxy derivative by the liver (2). Subsequently 25-OH-CC is converted to 1,25-$(OH)_2$-CC by the kidney (3). Thus a major portion of the time delay may be due to the obligatory metabolism of CC to its active

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[1]The abbreviations employed are: CC = cholecalciferol (vitamin D_3); 25-OH-CC = 25-hydroxycholecalciferol; 1,25-$(OH)_2$-CC = 1,25-dihydroxycholecalciferol.

form 1,25-$(OH)_2$-CC. However, another possible contributing factor to the lag in biological responses to CC may be a result of some as yet undefined induction process.

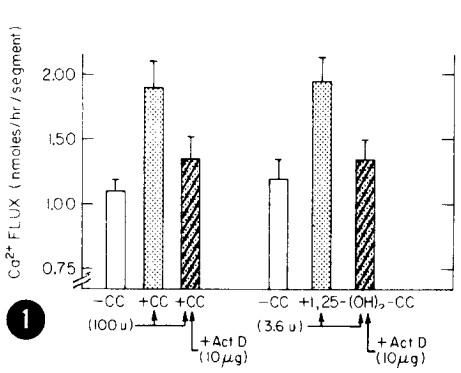
Norman (4, 5) and Zull et al.(6) have previously shown that actinomycin D given prior to CC inhibited the mediation of increased intestinal calcium absorption and elevated serum calcium levels. Also actinomycin D inhibited the stimulatory effects of CC on intestinal mucosal RNA synthesis (7). It is the purpose of this paper to further test the validity of the induction hypothesis by measuring the effect of the biologically active form of calciferol, 1,25-dihydroxy-cholecalciferol on the pulse labeling of intestinal mucosa RNA by ^{3}H-uridine and the effect of actinomycin D on 1,25-dihydroxy-CC stimulated Ca^{2+} transport and RNA synthesis.

Methods

Vitamin D deficient chicks were raised on a diet which has previously been described (8). 1,25-$(OH)_2$-CC (325 pmoles) was injected intracardinally in 0.2 ml of 1,2-propanediol. At varying time periods after 1,25-$(OH)_2$-CC administration, 1-2 μCi of ^{3}H-uridine (11.6 Ci/m mole, New England Nuclear) was injected intracardially in 0.2 ml of 0.15 M NaCl. Twenty minutes later the chicks were sacrificed. The small intestine was excised and washed in ice cold 0.25 M sucrose in 0.01 M Tris-HCl, pH 7.5, 0.025 M KCl and 0.005 M $MgCl_2$. The mucosa was scraped from the serosa with the aid of microscope slides, and then wrapped in aluminum foil and frozen immediately.

RNA was isolated by a procedure similar to that used by Hiat (9) or Scherrer and Darrell (10) as described by Norman et al. (7).

Twenty mg of dried nucleic acid were dissolved in 1.5 ml of 0.5 N NaOH (overnight at 37°C). Two 0.5 ml aliquots were removed for liquid scintillation counting. The purity of the isolated nucleic acid was assessed by determination of its U.V. absorbance spectrum. The absorbance at 260 nm was also determined for each sample (ranging from 8-10 X 10^3 units per 0.5 ml sample), and the results are reported as dpm of tritium/absorbance unit X 10^4.



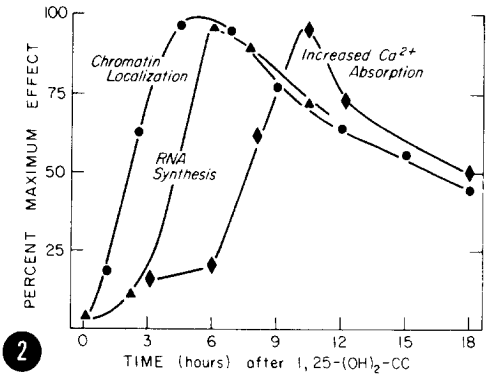


Figure 1. Effect of actinomycin D on CC or 1,25-$(OH)_2$-CC stimulation of calcium transport. The +CC chicks received a dose of 6.5 n moles of CC 23 hours before death and the actinomycin D (10 μg) was administered 3 hours before the CC. The + 1,25-$(OH)_2$-CC chicks received 234 pmoles of 1,25-$(OH)_2$-CC 9 hrs before death and the actinomycin D (10 μg) was administered in total of 4 doses 12, 10, 8 and 6 hrs prior to death.

Figure 2. Chronology of events which occur in the intestinal mucosa after administration of 1,25-$(OH)_2$-CC. Five units (325 pmoles) of either radioactive or non-radioactive 1,25-$(OH)_2$-CC were given to separate groups of rachitic chicks. Then the localization of 1,25-$(OH)_2$-CC in the intestinal chromatin, the stimulation of pulse labeling of RNA by ^3H-uridine and stimulation of the intestinal absorption of Ca^{2+} studied and each was expressed as the percent of the maximum observable effect.

The intestinal transport of $^{45}Ca^{2+}$ was measured *in vitro* exactly as described by Tsai *et al.* (11). The results are expressed as nmoles of Ca^{2+} transported per hour per ileal segment from the mucosal to the serosal side of the tissue (Figure 1). The intestinal absorption of $^{45}Ca^{2+}$, *in vivo* (Figure 2) was carried out as described by Norman (4). The subcellular localization of radioactivity in the intestinal chromatin fraction (Figure 2) after intracardial doses of 325 pmoles of 1,25-$(OH)_2$-[26,27-^3H]-CC was done exactly as described by Tsai *et al.* (11).

Results and Discussion

In Fig. 1 is shown the results of the blocking effect of actinomycin D on CC or 1,25-$(OH)_2$-CC stimulated Ca^{2+} transport. The prior administration of actinomycin D prevents the characteristic increase of intestinal calcium absorption which is mediated by both CC and 1,25-$(OH)_2$-CC. The effect of 1,25-$(OH)_2$-CC and actinomycin D on RNA synthesis was measured by pulse labeling

TABLE I. The effect of 1,25-dihydroxycholecalciferol and actinomycin D on RNA labeling by ^{3}H-uridine.

A.

Time between 1,25-(OH)$_2$-CC and ^{3}H-uridine (hours)	dpm/O.D. units Experiment A	Experiment B
-D Control	390 ± 15	490 ± 20
1/2	340 ± 10	---
1	320 ± 15	520 ± 10
2	350 ± 15	480 ± 10
4	315 ± 10	850 ± 20
6	---	820 ± 15
7	630 ± 5	---
10	---	610 ± 15

The experimental protocol is described under methods. Each number is the average of 4 determinations ± the standard deviation on 2 pairs of chicks.

B.

Treatment	dpm/O.D. units Experiment A	Experiment B
-D Control	1580	1640
+1,25-(OH)$_2$-CC (0.325 nmoles)	1900	3300
+1,25-(OH)$_2$-CC, + Actinomycin D (0.325 nmoles)	370	1200
-D, + actinomycin D	270	1100

1,25-(OH)$_2$-CC was injected intracardially 6 hrs before the ^{3}H-uridine. Actinomycin D was injected intraperitoneally (20 μg/100 gm chick) 1 hr before 1,25-(OH)$_2$-CC and again (10 μg/100 g chick) 3 hrs after the 1,25-(OH)$_2$-CC. Each number is the average of duplicate determinations from 3-4 chicks.

of intestinal mucosa RNA with ^{3}H-uridine as shown in Table 1A and B. After administration of 1,25-(OH)$_2$-CC, there is a two-fold increase in the rate of

RNA labeling. The maximum stimulation occurred within 6 hrs after the administration of the 1,25-$(OH)_2$-CC. As shown in Table 1B, the stimulatory effect of 1,25-$(OH)_2$-CC on RNA synthesis was also markedly inhibited by actinomycin D. These results are consistent with the suggestion that the primary biochemical response of the intestinal mucosa to CC is the initiation of RNA and protein synthesis.

We have previously reported that after a physiological dose of 1,25-$(OH)_2$-CC, that the majority of radioactivity isolated from target tissue intestine was localized in the nuclei and its chromatin fraction (11). Furthermore, this radioactivity was present in the form of 1,25-$(OH)_2$-CC. We have also found that the association of 1,25-$(OH)_2$-CC with the intestinal nucleus and its chromatin fraction precedes the development of the physiological responses (11). In Fig. 2 is shown the chronology of events which we have found to occur after the administration of 1,25-$(OH)_2$-CC. The maximum localization of 1,25-$(OH)_2$-CC in intestinal chromatin occurs 4 hrs after the administration of 1,25-$(OH)_2$-CC. This is followed by the stimulation of RNA synthesis which reaches a maximum at 6 hrs. Then, the maximum stimulation of intestinal Ca^{2+} transport occurs at 9 hrs after administration of 1,25-$(OH)_2$-CC.

The data presented in this paper demonstrate a direct stimulatory effect of 1,25-$(OH)_2$-CC on RNA synthesis and strongly suggest that the mode of action of 1,25-$(OH)_2$-CC is very similar to other steroid hormones. That is as a consequence of the association of 1,25-$(OH)_2$-CC and/or its receptor with the nucleus of the target tissue, there ensues an activation or stimulation of the biochemical expression of genetic information, which is utilized to initiate the physiological responses characteristic to CC. Much further work remains to precisely define the biochemical nature of these steps.

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