STUDIES ON THE MECHANISM OF ACTION OF CALCIFEROL VII. LOCALIZATION OF 1,25-DIHYDROXY-VITAMIN D_3 IN CHICK PARATHYROID GLANDS

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Summary

When 1,25$(OH)_2$-vitamin D_3 was administered to vitamin D-deficient chicks, within two hours the parathyroid glands were observed to accumulate this steroid to a concentration four times that present in the blood and equivalent to levels observed in the target intestine. Similarly, when 25-(OH)-vitamin D_3 was administered, the parathyroid glands had 2.4 times the concentration of the metabolite, 1,25-$(OH)_2$-vitamin D_3 as that seen in the blood and 60% of that found in the intestine. These results are consistent with the concept that the hormonally active form of vitamin D, 1,25-$(OH)_2$-vitamin D_3, may interact with the parathyroid glands to effect changes in parathyroid hormone secretion.

The identification of 1,25-dihydroxy-vitamin D_3[1], the biologically active form of vitamin D_3 (cholecalciferol) as part of the endocrine system which regulates calcium metabolism (1,2) has led to much speculation as to the interaction of this steroid with another major component of the calcium homeostatic system, parathyroid hormone (PTH). Much evidence has been accumulated which suggests that PTH is capable of maintaining and increasing the renal production of 1,25-$(OH)_2$-D_3 (3-6). Furthermore, it has been demonstrated that dietary vitamin D and its active metabolite, 1,25-$(OH)_2$-D_3, have quantitative suppressive effects on the renal production of 1,25-$(OH)_2$-D_3 (3). On the basis of these and other observations, a model was proposed for the regulation of steady state levels of the kidney concentrations of 25-hydroxy-vitamin D_3-1-hydroxylase which emphasized the interaction of vitamin D status and PTH status in the determination of the levels of this

[1]Abbreviations employed are: 25-hydroxyvitamin D_3 (25-OH-D_3); 1,25-dihydroxy-vitamin D_3 (1,25-$(OH)_2$-D_3) and parathyroid hormone (PTH).

enzyme (3). There are many points at which this interaction might occur. One possibility is the parathyroid gland itself wherein the final metabolite, 1,25-$(OH)_2$-D_3, could exert a feedback effect, decreasing PTH secretion. This would have at least two effects: (a) a decrease in the stimulatory effect of PTH on the renal 1-hydroxylase, and (b) a decrease in the entry of calcium into the blood from the intestine (stimulated by 1,25-$(OH)_2$-D_3) and from bone (stimulated by both the steroid and PTH). To study the way in which the actions of these two hormones may interact, the possible localization of radioactively labelled cholecalciferol metabolites in the parathyroid glands and other tissues of the vitamin D deficient chick has been investigated.

Methods

25-Hydroxy-[26,27-^{3}H]-vitamin D_3 (1.09 Ci/mmole) was obtained from Amersham Searle. 1,25-Dihydroxy-[26,27-^{3}H]-vitamin D_3 (0.71 Ci/mmole) was prepared biosynthetically utilizing rachitic chick kidney homogenates as previously described (7).

White Leghorn cockerels were obtained on the day of hatching and fed a standard rachitogenic diet (8). They received daily oral doses of 3.2 nmoles cholecalciferol for fourteen days, at which time steroid administration was terminated. Chicks were used in their seventh or eighth week when they had become vitamin D deficient and hyperparathyroid. Two hours after an intracardial (IC) dose of 1,25-$(OH)_2$-^{3}H-D_3 (720 pmoles/bird) chicks were sacrificed and tissues removed and placed in ice-cold saline. Lipids were extracted according to the method of Bligh and Dyer (9).

The lipid extracts of tissues from chicks dosed with 25-OH-^{3}H-D_3 were chromatographed on Sephadex LH-20 columns (1.0 x 80 cm) with 35% hexane in chloroform as the elution solvent. Each sample was cochromatographed with ^{14}C-labelled vitamin D metabolites prepared from intestinal mucosal lipid extracts from 3-week old vitamin D-deficient chicks 16 hours after a 650 pmole dose of 4-^{14}C-cholecalciferol. 140 fractions of 3.0 ml were collected and air dried in scintillation vials. Radioactivity was determined in a Beckman LS-200 liquid scintillation counter and efficiency determinations were made through the use of an external standard.

Results

In the first experiment chicks were sacrificed two hours after receiving 1,25-$(OH)_2$-^{3}H-D_3. The average weight of the parathyroid glands was 78 mg/chick, which is consistent with the severe hyperparathyroid state known to occur with the hypocalcemia resulting from vitamin D deficiency. In Table I are shown the amounts of radioactivity found in each tissue studied and the concentration of 1,25-$(OH)_2$-D_3 it represents. Clearly, as shown by the last line of this table, the parathyroids are capable of concentrat-

TABLE I. Localization of 1,25-dihydroxy-vitamin D_3 in Parathyroid Glands and Other Tissues

	Blood	Kidney	Intestine	Parathyroid
dpm/gram tissue	520	870	2300	2200
pmoles/gram tissue	0.33	0.56	1.5	1.4
concentration (x 10^{-9}M)	0.44	0.75	2.0	1.9
concentration ratio to blood	1.0	1.7	4.5	4.3

Chicks were injected intracardially with 320 pmoles 1,25-$(OH)_2$-D_3 and two hours later the tissues were removed, lipids extracted and radioactivity determined as described in Methods.

ing 1,25-$(OH)_2$-D_3 at least 4-fold over circulating concentrations. They have nearly as high a level of the steroid as does the intestine, a major target organ known to contain specific binding proteins for 1,25-$(OH)_2$-D_3 (10, 11), and a higher level than bone, the other major target tissue of 1,25-$(OH)_2$-D_3 action (12).

In order to determine whether the parathyroid glands would also localize endogenously produced 1,25-$(OH)_2$-D_3 and to what extent other vitamin D metabolites might be taken up, chicks were given 720 pmoles 25-OH-^{3}H-D_3 and sacrificed 16 hours later. Tissue lipid extracts were chromatographed as described in Methods. The results are shown in Figure 1 and summarized in Table II. 25-OH-D_3 and 1,25-$(OH)_2$-D_3 peaks were identified by their co-migration with the ^{14}C-labelled steroids described in Methods. As expected, the blood had the highest 25-OH-D_3 concentration (3.5 x 10^{-9}M), with other tissues having only 7-20% this amount. Of the total vitamin D_3 metabolites present in the blood, 95% was 25-OH-D_3, whereas only 27% of intestinal metabolites migrated in this peak. The pattern and conconcentration of vitamin D metabolites seen in these tissues are comparable to those reported previously (7, 12). Radio-

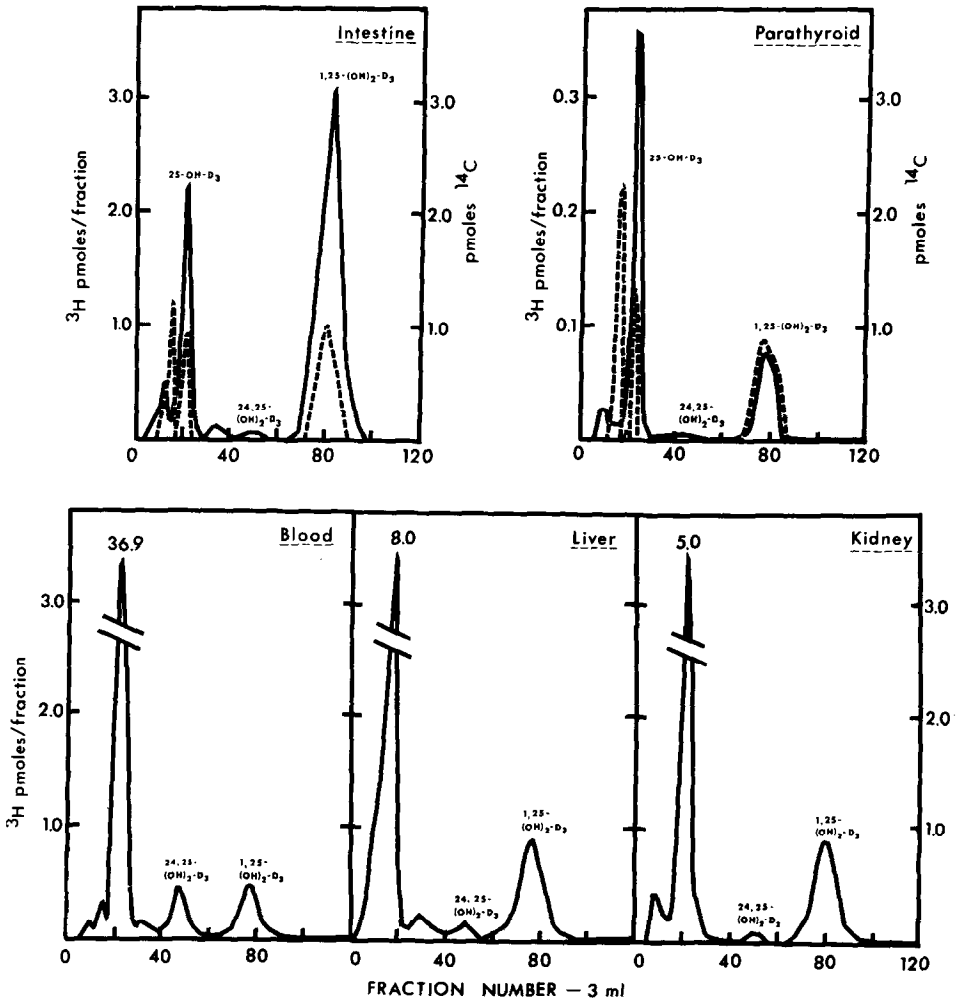

Figure 1. Localization of metabolites of 25-OH-^{3}H-D$_3$ in chick tissues. 16 Hours after an intracardial dose of 720 pmoles of 25-OH-^{3}H-D$_3$, lipids from the indicated tissues were extracted and chromatographed on Sephadex LH-20 columns as described in Methods. The pattern of ^{3}H metabolites obtained for each tissue is shown by the solid line. The dashed line indicates the elution position of ^{14}C-labelled D$_3$,25-OH-D$_3$ and 1,25-(OH)$_2$-D$_3$ standards prepared as described in Methods. These standards markers were run with each tissue lipid extract but were omitted from the lower three chromatograms for simplicity. In these three chromatographs the pmoles of 25-OH-^{3}H-D$_3$ in the peak fraction is indicated at the top.

activity migrating in the region of 24,25-(OH)$_2$-D$_3$ made up only a small proportion of the total amount recovered except in the case of the kidney in which it was 10% of the total. Of significance is the observation that the 1,25-(OH)$_2$-D$_3$ levels were highest in the intestine and parathyroid glands in

TABLE II. Localization of 25 Hydroxyvitamin D_3 and its Metabolites in Chick Tissues

A.	Total (fmoles/gram)	% Total 25-OH-D_3	24,25-$(OH)_2$-D_3	1,25-$(OH)_2$-D_3
Intestine	670	27	0.7	73
Parathyroid	820	63	1.7	35
Kidney	730	63	10	26
Liver	520	75	2.1	23
Blood	2700	95	0.6	4
B. Concentration (x 10^{-10}M)				
Intestine		2.4	0.07	6.6
Parathyroid		6.9	0.14	3.9
Kidney		6.1	1.0	2.5
Liver		5.2	0.15	1.6
Blood		34.0	0.21	1.6

Chicks were injected intracardially with 720 pmoles of 25-OH-^{3}H-D_3 and 16 hours later lipids were extracted from the indicated tissues and chromatographed as described in Methods (see Figure 1).

which the concentrations were 4.1 and 2.4 times that of the blood, respectively.

Discussion

The data reported here clearly indicate that in vitamin D-deficient chicks, the parathyroid glands are capable of accumulating 1,25-$(OH)_2$-D_3. This accumulation was observed both when rapid uptake of the exogenously administered steroid was measured and when its precursor was given, i.e.

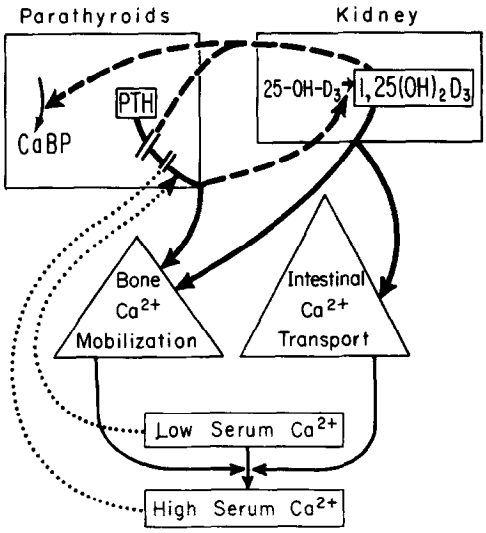

Figure 2. Interactions in calcium homeostasis. The heavy dashed line indicates the possible direct interactions between the parathyroid gland and the kidney. Arrows denote stimulation of a process and double bars indicate inhibition. Heavy solid lines represent the actions of the two calcium homeostatic hormones, PTH and 1,25-$(OH)_2$-D_3, on their target tissues which result in an increase in serum Ca^{2+} levels, as indicated at the bottom. The stimulatory and inhibitory effects of low and high serum calcium, respectively, on PTH secretion are shown by the dotted lines.

under circumstances when the steroid was produced endogenously. The rapidity of uptake, (first experiment) and concentrations of 1,25-$(OH)_2$-D_3 (both experiments) observed are similar to those reported for the other known target organs of vitamin D, the intestine (7) and the bone (12). Thus, these observations of selective accumulation of 1,25-$(OH)_2$-D_3 by the parathyroid against a concentration gradient suggest that this tissue also, like the intestine, may be a target of 1,25-$(OH)_2$-D_3 action.

The functions of 1,25-$(OH)_2$-D_3 in the parathyroid glands are not yet known, but two possibilities are indicated in Figure 2. In this figure, PTH is shown to increase serum calcium levels directly by stimulating bone mineral mobilization (13) and indirectly by maintaining or increasing the renal output of 1,25-$(OH)_2$-D_3 (3, 5). This steroid increases serum calcium levels through its action on the bone to stimulate calcium mobilization (12) and at the intestine to stimulate absorption of dietary calcium (8). In the parathyroid gland, 1,25-$(OH)_2$-D_3 may act to increase the level of the vitamin D

dependent calcium binding protein (CaBP) that has been reported to occur in this tissue (14) in much the same way as it stimulates CaBP synthesis in the intestine (15, 16) and kidney (17). The role of CaBP in the parathyroid gland is not clear, but it can be postulated to be involved in the regulation of PTH secretion by serum calcium levels (14, 18). Alternatively, or additionally, 1,25-$(OH)_2$-D_3 may, through its localization in the parathyroid glands, act as a feedback inhibitor of its own synthesis by reducing PTH secretion directly. Thus, in terms of bone mineral mobilization, a balance would be established between the two hormones capable of stimulating this process.

The crucial piece of evidence, of course, is whether 1,25-$(OH)_2$-D_3 does indeed decrease PTH secretion. Since a radioimmuno-assay for avian parathyroid hormone is not presently available, these experiments must be carried out in another species. Recent evidence suggests that 1,25-$(OH)_2$-D_3 does inhibit the output of PTH by bovine parathyroid glands (Prof. Anthony Care and Dr. David Baylink, personal communications). The elucidation of the interaction between 1,25-$(OH)_2$-D_3 and the parathyroid gland will contribute greatly to our understanding of the calcium homeostatic system.

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