Evidence for a new kidney-produced hormone, 1,25-dihydroxycholecalciferol, the proposed biologically active form of vitamin D

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Until recent years, little information has been available concerning the biochemical mode of action of vitamin D (calciferol) with regard to its important essential actions on calcium metabolism. This was due, on the one hand, to the extreme potency of the vitamin (1 international unit (IU) = 0.025 μg) and, on the other hand, to an inability to detect by direct chemical techniques amounts of the vitamin normally present in biological systems. However, with the advent of preparations of radioactive vitamin D₃ and new chromatographic techniques, it was possible to not only observe the conversion of the vitamin to a number of new metabolites, but also to study the tissue and subcellular localization of these individual metabolites. From such studies, it has now been established that vitamin D₃ undergoes several obligatory metabolic transformations prior to its affecting calcium metabolism in its primary target tissues, the intestine and bone.

Administration of radioactive vitamin D₃ to rachitic chicks or rats results in production of two major metabolites and a number of more minor metabolites (1, 2). The predominant metabolite present in the blood has been conclusively shown by DeLuca’s laboratory to be 25-hydroxyvitamin D₃ (3). It is known to be produced by the liver. In addition, two other relatively more minor metabolites have been isolated and characterized from porcine blood by DeLuca’s group. Their structures have been shown to be 21,25-dihydroxyvitamin D₃ and 25,26-dihydroxyvitamin D₃ (4, 5). The major metabolite of vitamin D₃ present in the target intestine has been known for several years to be chemically different from both the parent vitamin and 25-hydroxyvitamin D₃ (1, 2).

It has been coded metabolite 4B in my laboratory (1, 2), peak P in Kodicek’s laboratory (6), and peak V in DeLuca’s laboratory (7). The highly selective nature of the binding of metabolite 4B to the target intestine and the kinetics of appearance of this metabolite, in relation to the well-known lag in action of vitamin D-mediated calcium transport, originally suggested to us that metabolite 4B played a prominent role in the development of this physiological response (2). We have also found lower concentrations of metabolite 4B in bone and kidney of chicks, in the eggshell gland of the laying hen, other sites of intensive calcium metabolism, and in the intestinal mucosa of the frog, rat, rabbit, monkey, and man (8).

Although the intestine is by far the richest source of metabolite 4B, due to the low absolute amounts of the metabolite present (only some 4 to 6 pmoles or 2 to 3 ng per one chick intestine), it has proven virtually impossible to isolate enough metabolite 4B to permit its chemical characterization. Accordingly, a search was initiated for tissues that might be capable of producing metabolite 4B in an in vitro incubation. We had previously shown that the administration of radioactive 25-hy-


hydroxyvitamin D₃ to a rachitic chick resulted in the appearance and localization in the intestine of metabolite 4B (2). Thus 25-hydroxyvitamin D₃ was clearly an intermediate in the formation of metabolite 4B and could serve as a substrate in an in vitro incubation. Also, on the basis of chromatography of model compounds and the use of specifically labeled 1,2-³H vitamin D₃, it was known that metabolite 4B was more polar than 25-hydroxyvitamin D₃, probably by virtue of incorporation of a single polar functional group at the carbon-1 position (2, 9, 10).

Thus it has recently been reported by three laboratories (6, 11, 12) that the kidney is the major, if not exclusive, site of production of metabolite 4B. Further, we have shown that metabolite 4B produced from a kidney homogenate incubation is both chromatographically identical with and has biological activity equivalent to intestinal metabolite 4B (11). No evidence for the production of metabolite 4B was obtained from incubations of homogenates of liver, intestinal mucosa, bone, and adrenals with radioactive 25-hydroxyvitamin D₃.

This important finding has now opened the way to production via incubation in vitro of large enough quantities of metabolite 4B to permit its chemical characterization. Kodicek's group (13), DeLuca's group (14), and my laboratory (15) have all reported virtually simultaneously that the chemical structure of peak P, peak V, and metabolite 4B, respectively, is 1,25-dihydroxyvitamin D₃. The structure of this and several related compounds is given in Fig. 1. The metabolic step carried out by the kidney is the introduction of a single hydroxyl group at carbon-1. The stereochemistry of this hydroxyl is not yet definitely known. On the basis of metabolism, in vivo, of mixed doses of 1-α-³H-vitamin D₃ and 4-¹⁴C-vitamin D₃, in which it was found that there was a stereospecific loss of tritium, it seems likely that the new hydroxyl group is present in the α configuration.

Extensive studies have been carried out to determine the relative biological activities of vitamin D₃, 25-hydroxyvitamin D₃, and 1,25-dihydroxyvitamin D₃ (metabolite 4B) in terms of their ability to stimulate intestinal calcium transport (1, 2, 16). A summary of our results is given in Table 1. 1,25-Dihydroxyvitamin D₃ is four to five times as effective as vitamin D₃ and more than twice as effective as 25-hydroxyvitamin D₃ in stimulating intestinal calcium transport 24 hr after administration. If 1, 25-dihydroxyvitamin D₃ is the active form of the vitamin in the intestine and 25-hydroxyvitamin D₃ an intermediate in its formation, then 1, 25-dihydroxyvitamin D₃ should act considerably faster than either vitamin D₃ or its 25-hydroxyderivative. As shown in Fig. 2, this is the case. Following a considerable lag, vitamin D₃ and 25-hydroxyvitamin D₃ produce a maximum transport response only at 24 to 48 hr. Most significantly, 1,25-dihydroxyvitamin D₃ greatly shortens this lag, stimulating maximum calcium transport by 9 hr. At 9 hr, this metabolite is at least 13 times as active as the parent vitamin D₃. Haussler et al. (17) and Kodicek et al. (18) have also obtained results similar to this.

The other primary target organ for vitamin D action is the skeletal system. Although the exact biochemical function of the vitamin in this system is not clearly understood, one major action of vitamin D₃ here is to promote bone resorption to maintain normal blood calcium levels. Shown in Table 2 are the results of an experiment in which vitamin D₃, 25-hydroxyvitamin D₃, and 1,25-dihydroxyvitamin D₃ were tested for their ability to

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4 It should be noted that the α stereochemical designation given here refers to the stereochemistry of the C-1 tritium of the vitamin D₃ precursor, 7-dehydrocholesterol, which has an intact steroid B ring. In the correct stereochemical representation of the vitamin D molecule, as shown by X-ray crystallographic analysis, the A ring becomes inverted.
TABLE 1
The intestinal biological activities of 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃ relative to an equal amount of vitamin D₃.

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Relative activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin D₃</td>
<td>1.00</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D₃</td>
<td>1.78 ± 0.23 (5)</td>
</tr>
<tr>
<td>1,25-Dihydroxyvitamin D₃</td>
<td>4.74 ± 1.09 (4)</td>
</tr>
</tbody>
</table>

Numbers in parentheses are number of experiments.

The results were obtained from four or five experiments in which intestinal transport response was determined (16) 24 hr after oral administration of the metabolites. In this assay, the intestinal absorption of ⁴⁰Ca²⁺ is measured by sampling blood 30 min after administration of a dose of ⁴⁰Ca²⁺ + ⁴⁰Ca²⁺ in a duodenal loop. In each experiment, several standard levels of vitamin D₃ were also assayed. Then the responses of the test compound and vitamin D₃ were compared to determine a relative biological activity. Values are means ± the standard error of the mean.

stimulate bone calcium resorption (19). Under the conditions of this experiment, the only means by which the serum Ca²⁺ could be elevated was via bone resorption, as the birds were fed a “zero” Ca²⁺ diet. Of the three compounds tested, 1,25-dihydroxyvitamin D₃ was the most active. Also in analogy with the results shown in Fig. 1 for the intestinal response, 1,25-dihydroxyvitamin D₃ gave a greater bone resorption response at 8 hr than at 24 hr. Thus, 1,25-dihydroxyvitamin D₃ is the most rapid acting and biologically active compound in the two major target systems known to be responsive to vitamin D.

On the basis of these cumulative results obtained from several laboratories, it seems

TABLE 2
The bone resorption activity of vitamin D₃, 25-hydroxyvitamin D₃ and 1,25-dihydroxyvitamin D₃.

<table>
<thead>
<tr>
<th>Compound tested</th>
<th>Dose, IU</th>
<th>Serum Ca²⁺, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>125-D</td>
<td>24</td>
<td>6.4 ± 0.6</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>24</td>
<td>7.5 ± 1.1</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>47</td>
<td>8.1 ± 1.9</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D₃</td>
<td>11</td>
<td>7.4 ± 0.5</td>
</tr>
<tr>
<td>25-Hydroxyvitamin D₃</td>
<td>56</td>
<td>7.8 ± 1.1</td>
</tr>
<tr>
<td>1,25-Dihydroxyvitamin D₃</td>
<td>2</td>
<td>7.1 ± 0.7</td>
</tr>
<tr>
<td>1,25-Dihydroxyvitamin D₃</td>
<td>2</td>
<td>8.3 ± 1.3</td>
</tr>
</tbody>
</table>

The results are the average ± SD of groups of four to five rachitic chicks that received the test compound intracardially in 0.2 ml of 1,2-propanediol 24 hr prior to assay. The rachitic chicks had been placed on a “zero” calcium diet 4 days before administration of the test compound. The assay measures the elevation of serum Ca²⁺ that occurs as a result of bone resorption. Serum Ca²⁺ was determined by atomic absorption spectrophotometry.

FIG. 2. Time course of the intestinal calcium transport response. Each point represents the average of four to six chicks. Transport was assayed as described in (16). The compounds were administered orally. Open circles, 5 IU vitamin D₃; closed circles, 5 IU 25-hydroxyvitamin D₃; open triangles, 1.8 IU 1,25-dihydroxyvitamin D₃. Each point represents the average ± se for ⁴⁰Ca²⁺ absorption of three to five chicks.
logical to propose that 1,25-dihydroxyvitamin D₃ is a steroid hormone secreted by the kidney, which is then selectively accumulated by the intestinal mucosa. Here it exerts its characteristic effect on calcium metabolism. On this basis, vitamin D₃ and 25-hydroxyvitamin D₃ must be considered to be pro-hormones, at least for the production of 1,25-dihydroxyvitamin D₃. However, it is still possible that 25-hydroxyvitamin D₃ will prove to have independent biological effects in its own right, particularly in terms of mediating bone mineral mobilization. We have previously commented on the analogies in the mode of action of vitamin D₃ and that of several other steroid hormones, particularly aldosterone, testosterone, and estrogen (2, 19). A summary of the present understanding of the metabolism of vitamin D₃ is given in Fig. 3.

A major reason for the intense interest in development of the vitamin D metabolism story is due to the number of pathological conditions that might be related to abnormalities in the metabolism of the vitamin. These include vitamin D-resistant rickets, glucocorticoid antagonism of vitamin D action, sarcoidosis, and familial hypophosphatemia. It is particularly intriguing to consider the possible relationships that may exist between the kidney as a producer of the biologically active form of vitamin D and the osteomalacia and hypocalcemia associated with uremia.

It has been previously noted that an apparent resistance to the action of vitamin D exists in uremia (20). A number of laboratories (21, 22) have observed that in renal failure there is a negative calcium balance and a reduced absorption of calcium, which only in some instances can be alleviated by administration of very large amounts of vitamin D. Although Kimberg et al. (23), on the basis of studies with a uremic animal model, propose that renal insufficiency affects calcium transport in a manner independent of an interaction with vitamin D or its metabolites, Avioli et al. (24) support the concept that the metabolism of vitamin D is altered in chronic uremia. An obvious possibility is that a uremic kidney is unable to synthesize adequate amounts of 1,25-dihydroxyvitamin D₃ and, accordingly, intestinal calcium absorption becomes impaired and hypocalcemia results. It will be of crucial importance to carefully test for the presence of this possible metabolic defect and ascertain whether the pathological condition can be alleviated by administration of 1,25-dihydroxyvitamin D₃.

![Diagram of vitamin D metabolism]

**Fig. 3.** Summary of metabolism of vitamin D₃ (cholecalciferol).
There have been reports that the vitamin D resistance accompanying chronic renal failure may be more effectively overcome by administration of dihydroxycholesterol than vitamin D (25). Also, it has been shown in rats that dihydroxycholesterol may be converted to 25-hydroxydihydroxycholesterol in a manner analogous to the first hydroxylation of vitamin D. Presumably, this conversion also occurs in the liver. However, it is possible that the second kidney-mediated hydroxylation of 25-hydroxydihydroxycholesterol is not obligatory for biological activity. When the structures of 25-hydroxydihydroxycholesterol and 1,25-dihydroxyvitamin D₃ are compared (Fig. 1), it is apparent that due to the different conformation of the A ring of dihydroxycholesterol, as compared with vitamin D, that the 3-hydroxyl of 25-hydroxydihydroxycholesterol may be capable of mimicking the 1-hydroxyl of 1,25-dihydroxyvitamin D₃ in terms of satisfying the structural requirements of the receptors in the target organs. Thus, a possible explanation for the effectiveness of dihydroxycholesterol treatment in chronic renal failure is that due to its inverted A ring it may be able to bypass the metabolic transformation carried out by the kidney, which is essential for conversion of 25-hydroxyvitamin D to its biologically active form. Similarly, the other disease states mentioned will have to be carefully examined for possible defects in vitamin D metabolism. Certainly the prospect exists that the clinician will be able in the near future to consider new types of treatment of these various vitamin D-related disease states.

Summary

It has been conclusively shown that vitamin D₃ (cholecalciferol) must first be metabolized prior to its mediating intestinal calcium transport. The first transformation is conversion of vitamin D₃ by the liver to 25-hydroxyvitamin D₃. This compound is then subsequently metabolized by the kidney to 1,25-dihydroxyvitamin D₃. 1,25-Dihydroxyvitamin D₃ is over four times as effective as vitamin D₃ and more than twice as effective as 25-OH-vitamin D₃ in stimulating intestinal calcium transport. Additionally, 1,25-

dihydroxyvitamin D₃ is highly active in stimulating bone calcium resorption. As such, 1,25-dihydroxyvitamin D₃ likely represents the biologically active form of the vitamin in the intestine and bone. The secretion of this steroid by the kidney and its selective accumulation by the target organs supports the concept that this compound should be regarded as a hormonal regulator of calcium metabolism.

References


