Control of 25-Hydroxycholecalciferol Metabolism by Parathyroid Glands

(rat/vitamin D/parathyroid hormone/thyroparathyroidectomy/calcium)

M. GARABEDIAN, M. F. HOLICK, H. F. DELUCA*, AND I. T. BOYLE

Department of Biochemistry, College of Agricultural and Life Science, University of Wisconsin, Madison, Wis. 53706

Communicated by R. H. Burris, April 24, 1972

ABSTRACT Thyroparathyroidectomy of rats on a diet low in calcium reduces production of 1,25-dihydroxycholecalciferol from 25-hydroxycholecalciferol to negligible levels within 40 hr, and increases production of another metabolite, called Va. Parathyroid extract, at a dose of 20 units per day, prevents these changes. When 40 units per day of parathyroid extract is given 48 hr after thyroparathyroidectomy, 1,25-dihydroxycholecalciferol production is restored almost to control levels within 36 hr. The change brought about by parathyroid extract cannot be attributed to resulting changes in serum calcium or phosphorus concentration. It appears that the parathyroid hormone serves as a tropin for production of 1,25-dihydroxycholecalciferol, the hormonal form of vitamin D responsible for calcium mobilization from intestinal contents and bone.

Recently, a hormonal system involved in the regulation of calcium concentration in blood plasma was discovered. This system, which derives its active component from vitamin D, is located in kidney tissue. Vitamin D must first be metabolized in the liver to its 25-hydroxy derivative [25-hydroxycholecalciferol (25-OHD3)] (1-3). This metabolite is further converted in the kidney to either 1,25-dihydroxycholecalciferol [1,25-(OH)2D3] (4-7) or to an unidentified metabolite designated as Va (8-10). The Va metabolite has not been assigned a function, but evidence is convincing that 1,25-(OH)2D3 is the metabolically active form of vitamin D that induces intestinal calcium absorption (11-13) and mobilization of calcium from bone (14-16). The synthesis of 1,25-(OH)2D3 is regulated by negative feedback, thus establishing 1,25-(OH)2D3 as a true hormone involved in the regulation of the plasma calcium concentration (17, 18). The nature of the feedback regulation, i.e., the sharp shut-off of 1,25-(OH)2D3 synthesis at serum calcium concentrations very close to normal, suggested that the regulation involves secretion of parathyroid hormone. We considered it possible that the parathyroid glands might actually detect low serum calcium concentrations, and that the hormone might actually regulate the synthesis of 1,25-(OH)2D3 in kidney. It is the purpose of this communication to demonstrate that parathyroid hormone is a major factor in the regulation of 25-OHD3 metabolism that was previously attributed to serum calcium concentration.

MATERIALS AND METHODS

Hormones. Parathyroid extract was a gift from the Eli Lilly Co., Indianapolis, Ind., while highly purified parathyroid hormone was purchased from Wilson Laboratories, Chicago, Ill. The hormone was dissolved in 1 mM acetic acid, to which was added gelatin (15%) and phenol (0.4%). The final concentration was 5 units of hormone per 0.05 ml.

The [26,27-3H]25-OHD3 was synthesized in this laboratory (19), while the 1,25-(OH)2D3 was prepared with chicken kidney homogenate (20).

Animals and Experimental Procedure. Weanling, male albino rats obtained from the Holtzman Co., Madison, Wis., were placed in individual hanging wire cages and were fed a purified low calcium diet (21) containing 0.02% calcium, 0.30% phosphorus, and no vitamin D. After 3 weeks, the rats weighed 80-100 g. Each rat received intraperitoneally 325 pmol of 1,25-(OH)2D3 in 0.05 ml of 95% ethanol at 48 and 24 hr, and again intrajugularly 4 hr before surgery; this treatment increases the calcium concentration in blood serum to 6.8 mg/100 ml, as compared with 4 mg/100 ml usually observed in rats fed a low calcium diet (17).

Thyroparathyroidectomy was performed surgically on most of the rats. Half of the remaining controls were sham operated. Both control and operated rats received 325 pmol of 1,25-(OH)2D3 intraperitoneally every 24 hr during the remainder of the experiment. The 1,25-(OH)2D3 prevented the parathyroidectomized animals from dying of tetany.

Where indicated thyroparathyroidectomized rats received 5 or 10 units of parathyroid extract or parathyroid hormone subcutaneously every 6 hr, starting 6 or 48 hr after surgery. Control rats received the appropriate solvent or parathyroid extract or parathyroid hormone. All rats received 325 pmol of [3H]25-OHD3 in 50 μl of 95% ethanol intravenously 12 hr before they were killed by decapitation. This protocol provides an in vivo measurement of either 1,25-(OH)2D3 or Va production (17).

Analytical Procedure. The following procedures were performed on each rat. The blood collected after decapitation was centrifuged to harvest the serum. Serum calcium concentrations were determined in 0.1% LaCl3 with an atomic absorption spectrophotometer (Perkin-Elmer model 214). Serum phosphorus concentration was measured colorimetrically (22).

Tap water was added to the remaining serum to bring the volume to 5 ml, then 15 ml of methanol-chloroform 2:1 was added. The mixture was allowed to stand overnight at 4°. 5 ml of chloroform was added, the mixture was shaken, and the two phases were allowed to separate. The chloroform phase was drawn off and the aqueous phase was re-extracted with 5 ml of chloroform. The combined chloroform extracts were

Abbreviations: 25-OHD3, 25-hydroxycholecalciferol; 1,25-(OH)2D3, 1,25-dihydroxycholecalciferol.

* To whom reprint requests should be addressed.
dried with a flash evaporator, dissolved in 0.3 ml of 65:35 chloroform–Skellysolve B (petroleum ether, boiling point 67–68°), and applied to a 2 × 15-cm column containing 12 g of Sephadex LH-20 equilibrated with the same solvent mixture (23). 200 ml of this solvent was sufficient to elute 90–105% of the total radioactivity applied to the column. The eluent was collected in 5-ml fractions. The solvent was evaporated with a stream of air and the residue was dissolved in toluene counting solution. Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer, model 3375.

The chromatographic profile (Fig. 1) showed two major peaks besides the unchanged 25-OHD₃. The metabolites more polar than 25-OHD₃ were designated as peak Va (eluted at 70 ml) and peak Vc (eluted at 115 ml), as reported by Holick and DeLuca (23). Peak Va is a new metabolite generated by the kidney; its structure is currently under investigation. Peak Vc has been positively identified as 1,25-(OH)₂D₃ (5).

FIG. 1. Sephadex LH-20 chromatographic profiles of chloroform extracts of blood serum from rats given 325 pmol of [³H]25-OHD₃ intrajugularly 12 hr before death. Thyroparathyroidectomy was performed 84 hr before, and the treatment with parathyroid extract was started 40 hr before death. Vc = 1,25-(OH)₂D₃; the identity of Va is unknown. Left, control; center, thyroparathyroidectomized; right, operated and treated with parathyroid extract.

As expected from previous results (17, 18), control or sham-operated rats fed the low calcium diet release mainly 1,25-(OH)₂D₃ (peak Vc) into their plasma (Fig. 1). However, thyroparathyroidectomy 40 hr before death almost completely eliminates the appearance of 1,25-(OH)₂D₃ and increases the production of Va. If parathyroid extract is administered beginning 6 hr after surgery, and administrations are continued at a dosage of 10 units every 6 hr, only 1,25-(OH)₂D₃ accumulates, with little Va evident. Therefore, parathyroid extract can substitute for the thyroid and parathyroid glands in maintenance of 1,25-(OH)₂D₃ production. Interestingly, the amount of parathyroid extract used did not bring serum calcium and phosphorus concentrations to control levels (Table 1), although it caused large changes in 25-OHD₃ metabolism. Apparently the changes are effected by some specific component(s) of the extract, rather than by a parathyroid extract-induced change in serum calcium, phosphorus, or calcium–phosphorus balance, per se (Table 1).

The change in 25-OHD₃ metabolism after thyroparathyroidectomy is shown in Fig. 2. Rats under this regime show definite tetany 6 hr after the operation, and about 30% of the animals succumb to tetany near 20 hr. Note that 24 hr after the operation the production of [³H]1,25-(OH)₂D₃ already has decreased 80%, and that at 48 hr the compound is virtually absent, while Va increases from 1.5% of the total radioactivity before surgery to 9.6% 84 hr after. If the rats receive 10 units of parathyroid extract 48 hr after the operation, and subsequently every 6 hr, the concentration of [³H]1,25-(OH)₂D₃ increases rapidly (Fig. 2). A high concentration is reached by 36 hr (84 hr, Fig. 2) after the initial treatment with parathyroid extract. Even at 12 hr (60 hr, Fig. 2), the 1,25-(OH)₂D₃ becomes the major metabolite, increasing from 0.5 to 4%, while Va drops from 6 to 3% of the radioactivity recovered from the chromatogram.

The relationship of dose of parathyroid extract to 1,25-(OH)₂D₃ production after thyroparathyroidectomy is shown in Figs. 3 and 4. Although a daily dose of 20 units is sufficient to maintain the control concentrations of Va and 67% of the control concentration of 1,25-(OH)₂D₃, 40 units/day is re-

FIG. 2. Effect of thyroparathyroidectomy and parathyroid extract on the rate of production of two polar metabolites of 25-OHD₃, Va (unknown) and Vc [1,25-(OH)₂D₃] in the blood are shown as % of the total radioactivity recovered from the column of Sephadex LH-20. The rats received 325 pmol of [³H]25-OHD₃ intrajugularly 12 hr before death. Half of the rats received 10 units of parathyroid extract (second vertical bars) 48 hr after the operation (first vertical bars) and 10 units every 6 hr thereafter.

Each point is the mean ± standard deviation of the mean of 5 rats. [³H] in Va with (---) or without (-----) parathyroid extract; [³H] in Vc with (· · ·) or without (-----) parathyroid extract.

FIG. 3. Maintenance of the 1,25-(OH)₂D₃ (Vc) concentration by parathyroid extract administration to operated rats. The concentrations of metabolites are expressed as % of the total radioactivity recovered after chromatography of blood serum extract. (a) control; thyroparathyroidectomized rats received 5 (b) or 10 (c) units of parathyroid extract every 6 hr, starting 6 hr after surgery. Shaded, % of total recovered in Va; open, % of total recovered in Vc.


**Table 1. Effects of thyroparathyroidectomy and parathyroid extract on serum calcium, phosphorus, and metabolites of 25-OHDC**

<table>
<thead>
<tr>
<th></th>
<th>Control (11)</th>
<th>Control + parathyroid extract (3)</th>
<th>Operated (6)</th>
<th>Operated + parathyroid extract (4)</th>
<th>Thyroidectomized† (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum calcium (Ca,) mg/100 ml</td>
<td>6.8 ± 0.1*</td>
<td>6.8 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>Serum phosphorus (P), mg/100 ml</td>
<td>8.9 ± 0.1</td>
<td>10.1 ± 0.1</td>
<td>11.5 ± 0.2</td>
<td>11.4 ± 0.1</td>
<td>10.9 ± 0.2</td>
</tr>
<tr>
<td>Product Ca × P (%)</td>
<td>61</td>
<td>68</td>
<td>50</td>
<td>51</td>
<td>65</td>
</tr>
<tr>
<td>% of total radioactivity as V;</td>
<td>1.6 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>9.6 ± 0.8</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>% of total radioactivity as 1,25-(OH)₂D₃</td>
<td>12.6 ± 0.1</td>
<td>15.4 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>12.2 ± 0.6</td>
<td>16.0 ± 0.1</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean; (n) is number of rats in each group. The values were obtained 48 hr after surgery. Treatment with parathyroid extract (40 units/day) was started 40 hr after an operation; [H]25-OHDC was given 12 hr before death.
† Rats were considered thyroidectomized as described in the text.

required to restore 1,25-(OH)₂D₃ to control concentrations if administration is begun 48 hr after the operation (Fig. 4).

The figures and tables do not record the important fact that 20 units/day of highly purified parathyroid hormone had the same effect as the same dose of parathyroid extract. Thus, parathyroid hormone per se, and not some other component of the extract, is responsible for the effects observed in 25-OHDC metabolism.

Thyroid glands do not seem important to the responses noted (Table 1). Rats with a serum calcium concentration higher than 5 mg/100 ml and a phosphorus concentration lower than 11 mg/100 ml 2 days after surgery were considered thyroidectomized, but incompletely parathyroidectomized. In this group, 25-OHDC metabolism is similar to the control and the control + parathyroid extract.

It is interesting (Table 1) that treatment of normal rats with parathyroid extract (40 units/day) enhances the accumulation of 1,25-(OH)₂D₃ relative to the control rats, despite the already high concentration of circulating parathyroid hormone that can be expected with this low calcium diet.

Table 2 reveals the fact that kidney concentrations of V; and 1,25-(OH)₂D₃ reflect the same changes observed in plasma. This is not surprising, because both metabolites arise from the kidney (6, 8, 9). The intestinal mucosa also shows the decrease in 1,25-(OH)₂D₃ concentration after parathyroidectomy and the increase after parathyroid extract treatment. V; concentrations remained low in intestine under all circumstances tested.

**DISCUSSION**

It is evident that the parathyroid gland plays an important role in regulation of the metabolism of 25-OHDC, primarily in controlling the conversion to 1,25-(OH)₂D₃, the form of the vitamin active in increasing intestinal calcium transport and in increasing mobilization of calcium from bone. The results suggest that parathyroid hormone may serve as a tropic hormone that ultimately stimulates 1,25-(OH)₂D₃ synthesis. Parathyroid hormone must be considered essential to the functioning of physiologic amounts of vitamin D₃; this conclusion is strongly supported by the well-known requirement for massive amounts of vitamin D for animals that lack parathyroid hormone.

Exactly how parathyroid hormone regulates 25-OHDC metabolism remains unknown. The results presented suggest that parathyroid might attach to specific sites in kidney to activate adenylate cyclase, as has been demonstrated by Chase and Aurbach (24). The cyclic AMP formed may stimulate production of the enzyme 25-OHDC-1-hydroxylase, which in turn produces 1,25-(OH)₂D₃. Recent results suggest that the 25-OHDC-1-hydroxylase enzyme is turned over very rapidly, with a half-life of about 2.5 hr, and that its messenger has a half-life of about 6 hr (Tanaka and DeLuca, unpublished results). It seems likely that parathyroid hormone stimulates synthesis of the 25-OHDC-1-hydroxylase enzyme, although further experiments are required to support such a conclusion.

These studies focus attention upon the kidney as an endocrine organ for secretion of the hormonal form of vitamin D responsible for the mobilization of calcium from bone and intestine. In many respects this system resembles the glucocorticoid system of the adrenals. Because parathyroid hormone acts as the vitamin D trophic hormone, one can legitimately ask the question: how much of the systemic effect of the hormone in the maintenance of plasma calcium concentration can be attributed to its control of the synthesis of 1,25-(OH)₂D₃? A major role of parathyroid hormone in the sustained regulation of serum calcium, at the expense of bone and possibly intestine, may well be at the level of regulation of synthesis of 1,25-(OH)₂D₃.

![FIG. 4. Relationship of dose of parathyroid extract to appearance of 1,25-(OH)₂D₃ (Vc) in blood of control rats (a) or rats thyroparathyroidectomized 8 hr earlier (b) and treated with 5 (c) or 10 (d) units of parathyroid extract every 6 hr, beginning 40 hr after the operation. Shaded, Va; open Vc.](image-url)
TABLE 2. Metabolites of [3H]25-OHD3 in various tissues of thyroparathyroidectomized rats with and without treatment with parathyroid extract

<table>
<thead>
<tr>
<th>Control</th>
<th>Operated</th>
<th>Operated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pmol/g</td>
<td>pmol/g</td>
</tr>
<tr>
<td>Blood serum</td>
<td>1,25-(OH)2D3</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1,25-(OH)2D3</td>
<td>1.12 ± 0.20</td>
</tr>
<tr>
<td>Kidney</td>
<td>1,25-(OH)2D3</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>1,25-(OH)2D3</td>
<td>0.16 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>1,25-(OH)2D3</td>
<td>0.06 ± 0.05</td>
</tr>
</tbody>
</table>

All values represent the mean ± standard error of 3 rats 84 hr after an operation and 12 hr after injection of 325 pmol of [3H]25-OHD3. Control rats were sham operated.

* Rats thyroparathyroidectomized with parathyroid extract treatment (40 units/day) starting 40 hr after surgery. The procedure used to extract the chloroform-soluble components of kidney and small intestine has been described (23).

The role of parathyroid hormone in the stimulation of 1,25-(OH)2D3 synthesis has interesting clinical implications. In the absence of this hormone, animals or man do not respond to normal amounts of vitamin D; massive amounts of vitamin D produce little effect on the serum calcium concentration of some severely hypoparathyroid patients. These clinical observations may be understandable on the basis that the hormone is essential for normal production of the form of vitamin D active in calcium metabolism. Thus, 1,25-(OH)2D3, or a close analog, may be useful in the treatment of hypoparathyroid patients. However, massive doses of vitamin D and smaller, but nevertheless large, doses of 25-OHD3 are known to raise the serum calcium concentrations of hypoparathyroid patients (25). Two possible explanations are: (i) there is sufficient parathyroid hormone even in hypoparathyroid patients to produce some conversion of 25-OHD3 to 1,25-(OH)2D3, or (ii) massive amounts of vitamin D can serve as a poor substitute for 1,25-(OH)2D3 in the stimulation of intestinal calcium transport.

Finally, it appears that parathyroid glands serve as the sensing organ for plasma calcium concentration in the calcium homeostatic mechanism. This gland, in response to hypocalcemia, secretes parathyroid hormone, which is transferred to the bone, kidney, and possibly other tissues. It is well established that parathyroid hormone has a direct action in the mobilization of calcium from the bone. It had been believed that the sole function of the hormone in kidney is to bring about a phosphate diuresis. Now, it appears that this hormone performs an important additional function in stimulating production of 1,25-(OH)2D3. This hormonal form of vitamin D3 is then transferred in the plasma to the intestine and bone, where it effects the mobilization of calcium.

This work was supported by grants from the USPHS, AM-14851 and AM-15512. I.T.B. received a Wellcome Research travel grant.