Vitamin D Status Regulates 25-Hydroxyvitamin D_3-1α-Hydroxylase and Its Responsiveness to Parathyroid Hormone in the Chick

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Abstract

We asked this question: Under normal or near-normal metabolic conditions, does the prevailing normal or near-normal vitamin D status dampen the activity of 25-hydroxyvitamin-D_3-1α-hydroxylase (1α-hydroxylase) such that it determines not only its "basal" activity but also its responsiveness to stimulation by increased circulating concentrations of parathyroid hormone (PTH)? To answer this question, we measured the activity of 1α-hydroxylase in chicks, with and without administration of PTH, immediately before and during deprivation of vitamin D. Before deprivation of vitamin D, 1α-hydroxylase activity increased only slightly with administration of PTH. With deprivation of vitamin D for 5 and 10 d, while the plasma concentrations of calcium and phosphorus persisted normal and unchanged, 1α-hydroxylase activity not only increased progressively but also became sharply and increasingly responsive to stimulation by administration of PTH. But after 15 d of vitamin D deprivation, and the supervention of hypocalcemia, 1α-hydroxylase activity was not further increased by the administration of PTH. With deprivation of vitamin D, the progressive increase in 1α-hydroxylase correlated inversely with circulating levels of 1,25-dihydroxyvitamin D (1,25-$[OH]_2D$), and the decreasing calcemic response to PTH correlated inversely with the responsiveness of 1α-hydroxylase to PTH (in chicks deprived of vitamin D for 1–10 d).

These results demonstrate that: under normal metabolic conditions, the normal vitamin D status regulates the activity of 1α-hydroxylase so as to dampen both its "basal" activity and its responsiveness to stimulation by PTH; and vitamin D deprivation insufficient to cause hypocalcemia enhances both the "basal" activity of 1α-hydroxylase and its responsiveness to stimulation by PTH. The results suggest that the normal dampening of 1α-hydroxylase and both of the demonstrated enhancements of its activity are mediated by normal and reduced levels of circulating 1,25-$(OH)_2D$, respectively. The finding that PTH fails to further stimulate 1α-hydroxylase when vitamin D deprivation is sufficient in duration to cause hypocalcemia confirms the findings of other investigators and again demonstrates that observations made during abnormal metabolic circumstances may bear little on the physiologic regulation of 1α-hydroxylase under normal or near-normal metabolic circumstances.

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Received for publication 19 March 1984 and in revised form 27 June 1984.

J. Clin. Invest.

0021-9738/85/01/0155/07 $1.00
Volume 75, January 1985, 155–161

Introduction

1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$)[1] is the metabolite of vitamin D currently considered to be the most biologically active with respect to bone resorption and intestinal absorption of calcium and phosphorus (1–4). The renal formation of 1,25-$(OH)_2D_3$ from its endogenous precursor, 25-hydroxyvitamin D_3 (25-OHD_3) is catalyzed by 25-hydroxyvitamin-D_3-1α-hydroxylase (1α-hydroxylase) (5–7), an enzyme whose activity can be suppressed by 1,25-$(OH)_2D_3$ (8–13) and stimulated by parathyroid hormone (PTH) (8, 10, 12–17). It has been proposed that this enzyme is regulated by a suppressive effect of 1,25-dihydroxyvitamin D (1,25-$[OH]_2D$) that countervails the stimulatory effect of PTH (8, 9, 12, 13). But it now seems clear that during dietary deprivation of calcium and phosphorus, normal and even increased plasma or renal cortical concentrations of 1,25-$(OH)_2D$ do not suppress 1α-hydroxylase activity, even when circulating levels of PTH are presumably not increased (18–22). Indeed, recently it has been found that 1,25-$(OH)_2D$ can stimulate the activity of 1α-hydroxylase, a finding that prompted Tanaka and Deluca (21) to state that ". . . a simple suppression or inactivation of the 1α-hydroxylase by 1,25-$(OH)_2D_3$ is no longer consistent with available data." In fact, evidence that 1,25-$(OH)_2D$ can countervail the stimulatory effect of PTH in vivo has been sought only during the unphysiological state of vitamin D deficiency in which hypocalcemia initially obtained, 1α-hydroxylase activity was increased, and plasma concentrations of 1,25-$(OH)_2D$ were greatly and abruptly increased by administration of pharmacologic amounts of 1,25-$(OH)_2D_3$ (10, 12). As others have pointed out (23, 24), observations made during such abnormal metabolic circumstances may bear little on the physiologic regulation of 1α-hydroxylase under normal or near-normal metabolic circumstances.

The question then remains: Under normal or near-normal metabolic conditions, when the prevailing circulating concentrations of 1,25-$(OH)_2D$, PTH, calcium, and phosphorus are continuously normal or near-normal, does the prevailing normal or near-normal vitamin D status dampen an otherwise greater activity of 1α-hydroxylase such that it determines not only its "basal" activity but also its responsiveness to stimulation by increased circulating concentrations of PTH? The results of the current study provide a yes answer to this question and suggest that with vitamin D deprivation insufficient to cause hypocalcemia, decreases in the plasma concentration of 1,25-$(OH)_2D$ not only increase the "basal" activity of 1α-hydroxylase, but also enhance its responsiveness to PTH.

1. *Abbreviations used in this paper:* 1,25-$[OH]_2D$, 1,25-dihydroxyvitamin D; 25-OHD_3, 25-hydroxyvitamin D_3; 1α-hydroxylase, 25-hydroxyvitamin-D_3-1α-hydroxylase; PTE, parathyroid extract; PTH, parathyroid hormone.

Methods

Animals. From hatching, white leghorn cockerels (H and N Hatchery, Petaluma, CA) were maintained on a vitamin D-deficient rachitogenic diet containing 0.5% calcium and 0.6% phosphorus (I. C. N. Products, Cleveland, OH). Vitamin D_3 (dissolved in Wesson Oil, Hunt Wesson Foods, Inc., Fullerton, CA), 20 IU daily, was administered orally for the first 3 wk of life, and then discontinued. In these chicks, the dietary supplement of 20 IU daily was deemed adequate, as evidenced by the presence of normocalcemia, normophosphatemia, and normal somatic growth: in 3-wk-old chicks supplemented with 20 IU vitamin D_3 daily, body weight was not different from that of 3-wk-old chicks supplemented with a greater amount of vitamin D_3, 100 IU daily (208±21 vs. 207±26 g, respectively). The effect of administered parathyroid extract (PTE) on 1α-hydroxylase activity was determined in chicks deprived of vitamin D for 1, 5, 10, and 15 d. Indwelling jugular catheters were placed under general anesthesia (6 mg/100 g body weight sodium pentobarbital [Abbott Laboratories, North Chicago, IL]) intramuscularly on the day before the study. PTE (Eli Lilly & Co., Indianapolis, IN), 15 U. S. Pharmacopeia U/100 g body weight, or vehicle alone (1.6% glycerin with 0.2% phenol) was administered intravenously through the jugular catheter every 2 h for four doses. 1 h after the last dose of PTE or vehicle, blood was drawn by intracardiac puncture for determination of plasma concentrations of calcium and phosphorus, the chicks were killed by decapitation, and the kidneys were removed rapidly and placed in an iced solution of 0.9% sodium chloride for determination of 1α-hydroxylase activity. In separate studies of chicks in which deprivation of vitamin D for 1, 5, 12, and 21 d was initiated at 3 wk (but to which vehicle or PTE was not given), blood was obtained by decapitation for determination of plasma concentration of 1,25-$(OH)_2$D, calcium, and phosphorus, and the kidneys were removed rapidly for determination of 1α-hydroxylase activity. Concentrations of 1,25-$(OH)_2$D were determined on plasma from single chicks when plasma obtained was sufficient, or pooled plasma obtained from 2 to 6 chicks. When pooling was necessary for a particular value of plasma concentration of 1,25-$(OH)_2$D, the reported values for plasma concentrations of calcium and phosphorus, and for 1α-hydroxylase activity, are the mean values obtained from all chicks whose plasma was pooled. In a separate group of chicks that received 100 IU vitamin D_3 three times a week from hatching for 3 wk and then were deprived of vitamin D for 21–56 d, blood was obtained by intracardiac puncture for determination of plasma concentrations of calcium and phosphorus, the chicks were killed by decapitation, and the kidneys were removed for determination of 1α-hydroxylase activity.

Enzyme assay. The kidney tissue was pressed through a prechilled tissue press; the expressed tissue was homogenized in 15 ml of an ice-cold solution containing 200 mM sucrose, 10 mM sodium malate, 2 mM magnesium chloride, and 15 mM Tris-HCl (pH 7.4) by grinding in a Potter-Elvehjem homogenizer (Potter Instrument Co., Inc., Plainview, NY) for three passes. A 3% (wt/vol) homogenate was prepared by addition of appropriate amounts of the above solution to the homogenate. 10 ml of the homogenate was set aside for determination of protein concentration by the Biuret method. 10 ml of the homogenate was preincubated for 1 min in a water bath at 37°C before initiation of the reaction by the addition of 1.7 nmol of 25-OH[26,27-^{3}H]D_3 (Amersham/Searle Corp., Arlington Heights, IL; sp act 161–183 dpm/pm) in 50 μl of ethanol. 4 min after adding substrate, the reaction was stopped by the addition of 50 ml of methanol/chloroform (2:1) to the incubation mixture. The 4-min time point was chosen because 1α-hydroxylase activity was found to be linear through 4 min (when measured at intervals of 1 min), and its extrapolated value to be zero at zero time. Enzyme activity became curvilinear after 5 min of incubation. The studies of those chicks that received 100 IU vitamin D_3 three times a week were performed earlier; specific activity of the 25-(OH)[26,27-^{3}H]D_3 was 27.8–29.6 dpm/pm, and an incubation period of 30 min rather than 4 min was used. Total lipids were extracted by a modified method of Bligh and Dyer (25). After evaporation of chloroform under nitrogen, the extracted lipids were redissolved in chloroform/hexane (65:35) for column chromatography.

Column chromatography. Column chromatography was performed as previously described utilizing Sephadex LH-20 column (1.0 × 60 cm) with chloroform/hexane (65:35) as solvent (16). 1,25-$(OH)_2D_3$ production is reported in picomoles per milligram protein per 4 or 30 min incubation. Column recovery of tritium was 98.7±5.0%. The homogeneity of the radioactive peak believed to be 1,25-$(OH)_2D_3$ was verified by co-migration with authentic 1,25-$(OH)_2D_3$ during high pressure liquid chromatography using a μm-porasil column with 10% isopropanol in hexane (26). There was no detectable 25,26-$(OH)_2D_3$.

1,25-$(OH)_2$D assay. Plasma concentrations of 1,25-$(OH)_2$D were measured by Dr. Bernard P. Halloran (Veterans Administration Medical Center, University of California, San Francisco, CA). Approximately 2,500 dpm each of chromatographically purified 25-(OH)[23,24(n)-^{3}H]D_3 and 1,25-$(OH)_2$[23,24(n)-^{3}H]D_3 (Amersham Corp., Arlington Heights, IL) in 0.02 ml of absolute ethanol were added to each 2-ml plasma sample to determine percentage of recovery. Plasma lipids were extracted twice with diethyl ether (3:1, vol/vol) and the extract was chromatographed on a column (0.7 × 12 cm) of Sephadex LH-20 developed in hexane/chloroform/methanol (9:1:1). The fraction from the LH-20 chromatography that contained 1,25-$(OH)_2$D was further purified by high-pressure liquid chromatography using two μm-porasil columns in series in a solvent system of hexane/isopropanol (9:1) at a flow rate of 2.0 ml/min. 1,25-$(OH)_2$D was quantitated in duplicate using a competitive protein binding assay modified from those described by Eisman et al. (27) and Shepard et al. (28). Intestinal cytosol binding protein was obtained from normal vitamin D-adequate chicks. Overall recovery of 1,25-$(OH)_2$D was 70–75%. Intraassay and interassay coefficients of variation were 7.7% and 11.6%, respectively. In normal adult subjects, the plasma concentration of 1,25-$(OH)_2$D was 33±3 pg/ml (n = 15).

Calcium, phosphorus. Plasma concentrations of calcium were determined by atomic absorption spectrophotometry. Plasma concentrations of inorganic phosphorus were determined by the Fiske-Subbarow method (29).

Statistical analysis. Statistical analysis was performed using unpaired t test. Slopes were determined by the method of least squares. Statistical significance of the linear regressions and the differences between slopes were assessed by analysis of covariance.

Results

Effect of vitamin D deprivation on 1α-hydroxylase activity and plasma concentrations of calcium and phosphorus. After the supplement of vitamin D was discontinued in the normally growing chicks, the activity of 1α-hydroxylase increased progressively as previously described (8, 11, 15): the enzyme activity increased significantly after deprivation of vitamin D for 5 d (Fig. 1) and before any changes in plasma concentrations of calcium or phosphorus had occurred. In chicks acutely deprived of vitamin D, a rise in enzyme activity has been documented to occur before the occurrence of hypocalcemia and hypophosphatemia (8, 15). After deprivation of vitamin D for 10 d, the activity tripled (Fig. 1). Frank hypocalcemia did not occur until the chicks were deprived of vitamin D for 15 d (Fig. 2). In chicks deprived of vitamin D for 1 d (and before administration of PTE or vehicle), plasma concentration of phosphorus was 5.1±0.6 mg/dl (mean±1 SD); plasma concentration of phosphorus became significantly reduced (4.3±0.3 mg/dl, $P < 0.001$ vs. 1 d) after 15 d of vitamin D deprivation.

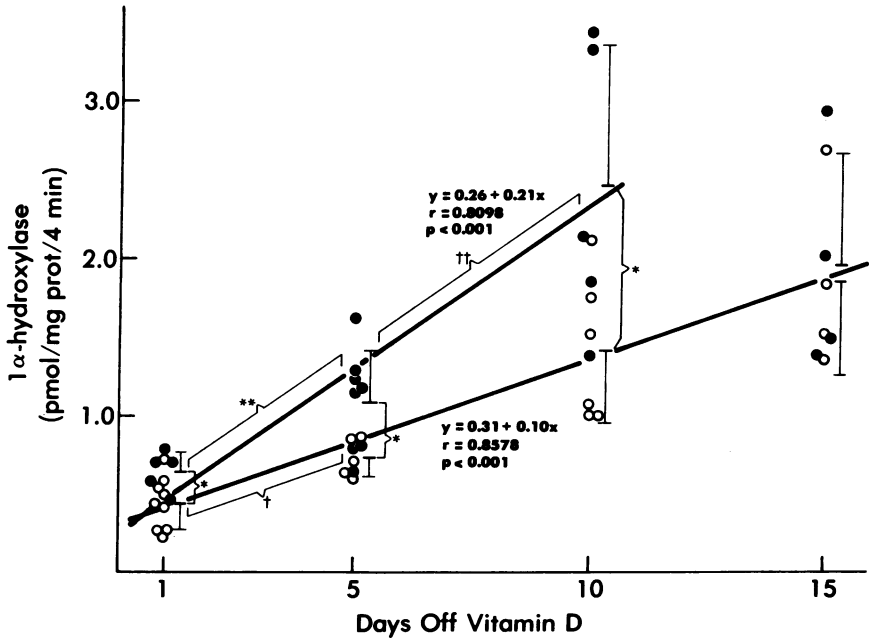

Figure 1. Relationship between 1α-hydroxylase activity and duration of deprivation of vitamin D in chicks given vitamin D 20 IU/d for 3 wk from hatching, then deprived of vitamin D for 1, 5, 10, and 15 d, and thereupon given either PTE (•) (15 USP U/100 g body weight) or vehicle (○) intravenously every 2 h for four doses. 1α-hydroxylase activity was measured 1 h after the last dose of PTE or vehicle. The shorter line was the best fit line calculated by the method of least squares for those chicks that received PTE and in which the mean activity of 1α-hydroxylase was higher than that of those chicks that received vehicle, specifically those chicks studied after 1, 5, and 10 d of vitamin D deprivation. The longer line was the best fit line calculated by the method of least squares for those chicks that received vehicle after 1, 5, 10, and 15 d of vitamin D deprivation. $^{*}P < 0.05$; $^{**}P < 0.02$; †$P < 0.01$; and ††$P < 0.002$.

Vitamin D deprivation and the response of 1α-hydroxylase activity to administration of PTE. With vitamin D deprivation, administration of PTE induced an increase in 1α-hydroxylase activity progressively greater than the increase induced by vitamin D deprivation alone: in chicks that received PTE, the slope of the regression line of 1α-hydroxylase activity plotted against the duration of vitamin D deprivation (0.21) was double that (0.10) of chicks that received vehicle alone ($P < 0.02$, Fig. 1). In the vitamin D-adequate chicks, administration of PTE induced only a slight increase in 1α-hydroxylase activity, an increase to a value not different from that obtaining in chicks not given PTE but deprived of vitamin D for 5 d, and a value lower than that obtaining in chicks not given PTE, but deprived of vitamin D for 10 d ($P < 0.01$). After 15 d of vitamin D deprivation, 1α-hydroxylase activity was not further increased by administration of PTE (Fig. 1). In a separate group of chicks that received 100 IU vitamin D_3 three times a week from hatching for 3 wk, and then were deprived of vitamin D for 21–56 d, 1α-hydroxylase activity did not correlate with plasma concentration of calcium (Fig. 3).

Vitamin D deprivation and the calcemic and phosphatemic response to administration of PTE. The calcemic response to PTE (as judged by plasma concentration of calcium after administration of PTE) decreased with progressive vitamin D deprivation ($r = 0.7452$, $P < 0.001$, Fig. 2) as previously described (30, 31). In the chicks that received PTE, the slope (0.29) of the regression line of plasma concentration of calcium plotted against duration of vitamin D deprivation was greater than that (0.11) of chicks that received vehicle ($P < 0.02$). By 15 d of vitamin D deprivation, administration of PTE no longer induced an increase in plasma concentration of calcium (Fig. 2). In chicks deprived of vitamin D for 1, 5, and 10 d,

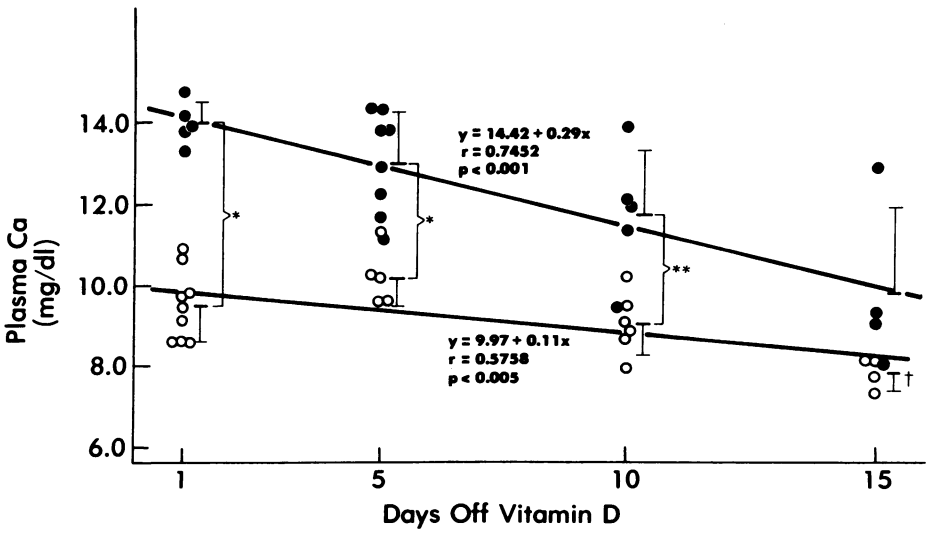

Figure 2. Relationship between plasma concentration of calcium and duration of deprivation of vitamin D in chicks given vitamin D 20 IU/d since hatching for 3 wk and then deprived of vitamin D for 1, 5, 10, and 15 d, and thereupon given either PTE (•) (15 USP U/100 g body weight) or vehicle (○) intravenously every 2 h for four doses. Plasma concentration of calcium was measured 1 h after the last dose of PTE or vehicle. $^{*}P < 0.001$; $^{**}P < 0.005$; and †vs. 1-d vehicle ($P < 0.005$).

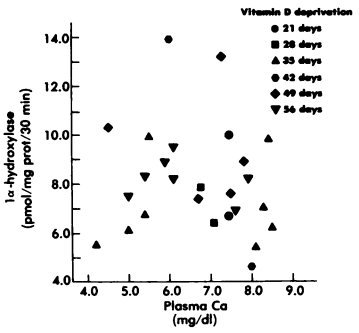

Figure 3. Relationship between 1α-hydroxylase activity and plasma concentration of calcium in chicks given vitamin D_3 100 IU three times a week for 3 wk and then deprived of vitamin D for 21–56 d.

the calcemic response to PTE correlated inversely with 1α-hydroxylase activity determined after administration of PTE ($P < 0.001$, Fig. 4).

In chicks deprived of vitamin D for 1 d, administration of PTE increased plasma concentration of phosphorus from 5.1±0.6 to 6.0±0.9 mg/dl ($P < 0.01$). In all other groups, plasma concentration of phosphorus did not change when PTE or vehicle was administered. In chicks deprived of vitamin D for 15 d, plasma concentration of phosphorus was greater in those chicks given PTE than in those chicks given vehicle (4.8±0.2 vs. 3.9±0.3 mg/dl, $P < 0.005$); in all other groups, plasma concentrations of phosphorus in those chicks given PTE were not different from those of chicks given vehicle.

Vitamin D deprivation and circulating levels of 1,25-(OH)$_2$D. In another group of chicks given 20 IU vitamin D_3 from hatching for 3 wk and then deprived of vitamin D, 1α-hydroxylase activity correlated inversely with the progressive decrease in circulating levels of 1,25-(OH)$_2$D that occurred with vitamin D deprivation ($r = 0.7884$, $P < 0.001$, Fig. 5). In this group of chicks, 1α-hydroxylase also correlated inversely with the plasma concentration of calcium ($r = 0.7450$, $P < 0.005$).

Discussion

The results of the current study demonstrate that when vitamin D status is normal or near-normal, the prevailing vitamin D

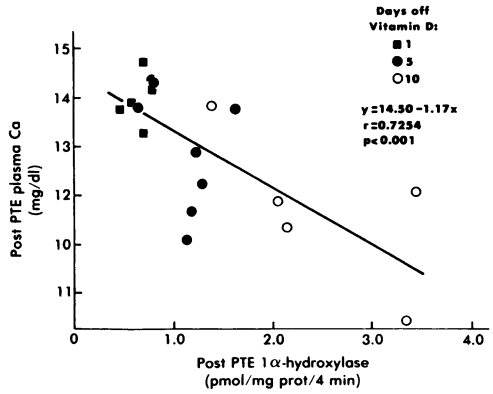

Figure 4. Relationship between plasma concentration of calcium and 1α-hydroxylase activity in chicks given vitamin D 20 IU/d since hatching for 3 wk, then deprived of vitamin D for 1, 5, and 10 d, and thereupon given PTE (15 USP U/100 g body weight).

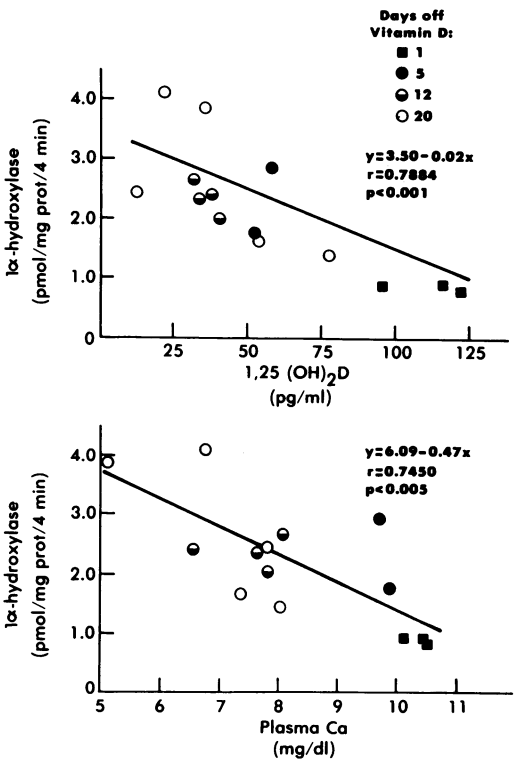

Figure 5. Relationship between 1α-hydroxylase activity and the circulating levels of 1,25-(OH)$_2$D (*top*), and the plasma concentration of calcium (*bottom*) in chicks given vitamin D 20 IU/d since hatching for 3 wk, and then deprived of vitamin D for 1, 5, 12, and 20 d.

status determines both the "basal" activity of 1α-hydroxylase as well as its responsiveness to stimulation by PTH: in the intact, normally growing 3-wk-old chick that has received a normal dietary supplement of vitamin D since hatching, "basal" 1α-hydroxylase activity is normally dampened, and the enzyme is only minimally responsive to stimulation by PTH. However, with deprivation of vitamin D for 5 and 10 d, the "basal" activity of 1α-hydroxylase not only increases progressively (8, 11, 15), but also becomes sharply and increasingly responsive to stimulation by PTE, even though the plasma concentrations of calcium or phosphorus remain normal and unchanged (in those chicks not given PTE).

In previous studies in which evidence has been sought that 1,25-(OH)$_2$D can countervail the stimulatory effect of PTH on 1α-hydroxylase activity in vivo, the nonphysiologic, hypocalcemic state of vitamin D deficiency obtained (10, 12). Interpretation of these studies is difficult, since hypocalcemia per se can stimulate 1α-hydroxylase activity (as judged by increased circulating levels of 1,25-(OH)$_2$D in the absence of PTH [32]), can enhance stimulation of the enzyme by PTH (32), may prevent 1,25-(OH)$_2$D from suppressing its own production (12, 13, 21), and, indeed, may even permit 1,25-(OH)$_2$D to stimulate its own production: in rats fed a low calcium, vitamin D-deficient diet, provision of progressively greater amounts of vitamin D, which induced progressive increases in circulating levels of 1,25-(OH)$_2$D, was attended by progressive increases in 1α-hydroxylase activity despite a progressively less severe hypocalcemia, and a presumably less severe hyperparathyroidism (21). In rats fed a low calcium, vitamin D-deficient diet,

provision of 1,25-$(OH)_2D_3$ more than doubled the activity of 1α-hydroxylase activity despite mitigating the attendant hypocalcemia (21). And in humans with vitamin D deficiency treated with vitamin D, the finding that supernormal circulating levels of 1,25-$(OH)_2D$ occur and persist while hypocalcemia persists (33–36), presumably reflects a greatly increased activity of 1α-hydroxylase. Clearly, 1,25-$(OH)_2D$ does not always dampen 1α-hydroxylase activity. Rather, depending upon the prevailing metabolic circumstances, 1,25-$(OH)_2D$ can either dampen or enhance this activity. Specifically, the published observations suggest that the capacity of 1,25-$(OH)_2D_3$ to dampen 1α-hydroxylase activity may depend upon the prevailing serum concentration of calcium (and phosphate), and possibly even the duration of a given concentration of calcium or the range of its concentrations (22, 23, 37).

The current study suggests that during normal metabolic circumstances, the capacity of 1α-hydroxylase to be stimulated by even greatly increased circulating levels of PTH is constrained. Such constraint would restrict increases in circulating levels of calcium that might otherwise occur when circulating levels of PTH increase during normal physiologic perturbations. However, with minimal deprivation of vitamin D, and before a frank decrease in plasma concentration of calcium occurs, increased responsiveness of 1α-hydroxylase to stimulation by PTH would enhance conversion of 25-OHD to 1,25-$(OH)_2D$. This enhancement would tend to maintain the plasma concentration of 1,25-$(OH)_2D$ at a value higher than would otherwise obtain, and thus postpone or prevent the occurrence of hypocalcemia.

Clearly, 1,25-$(OH)_2D$ can suppress the activity of 1α-hydroxylase (8–13) and enhance the bone resportive response to PTH (38, 39). In the currently studied chicks, the progressive increase in "basal" activity of 1α-hydroxylase that occurred with deprivation of vitamin D correlated inversely with the progressive decrease in circulating levels of 1,25-$(OH)_2D$. And, the progressive decreasing calcemic response to PTE that occurred with deprivation of vitamin D correlated inversely with the progressive increasing responsiveness of 1α-hydroxylase to administration of PTE. In experimental vitamin D deficiency (40–43) and in renal insufficiency (27, 44), the reduced circulating concentrations of 1,25-$(OH)_2D$ would appear to account for at least some of the impaired calcemic response to PTH, since in both conditions the impairment can be largely corrected by administration of 1,25-$(OH)_2D$ (38, 39). Normal plasma concentrations of 1,25-$(OH)_2D$ could then account for the normally dampened "basal" activity of 1α-hydroxylase and its constrained responsiveness to stimulation by PTH. And, decreasing plasma concentrations of 1,25-$(OH)_2D$ could account for both the increasing "basal" activity of 1α-hydroxylase and its increasing responsiveness to PTH that attended continued deprivation of vitamin D before hypocalcemia supervened.

It is clear that in hypocalcemic vitamin D deficiency, PTH contributes to the increased activity of 1α-hydroxylase, since parathyroidectomy is associated with a large reduction in 1α-hydroxylase activity (8, 10, 14–16). But, in the currently studied chicks with severe hypocalcemic vitamin D deficiency induced by deprivation of vitamin D for 15 d, the already-increased activity of 1α-hydroxylase was not further increased by administration of PTE, and in chicks deprived of vitamin D for 21–56 d, 1α-hydroxylase activity was not greater in the more hypocalcemic, and therefore presumably more hyperparathyroid (45, 46) chicks than in those chicks with less severe hypocalcemia. Thus, while PTH contributes to the greatly increased activity of 1α-hydroxylase characteristic of the hypocalcemic vitamin D-deficient state, above some level of circulating PTH, further increases do not further increase the activity of the enzyme. Consistent with this formulation is the observation that in frankly vitamin D-deficient rats, apparent 1α-hydroxylase activity did not increase further with increasing severity of hypocalcemia (47). In intact vitamin D-deficient rats, administration of synthetic bovine 1-34 amino-terminus PTH did not further stimulate 1α-hydroxylase activity in the proximal convoluted tubule (48). And, in vitamin D-deficient rats in which administration of PTE from the time of thyroparathyroidectomy prevented the otherwise predictable decrease in apparent 1α-hydroxylase activity, a threefold increase in the dose of PTE administered did not induce a further increase in apparent 1α-hydroxylase activity even though this dose was sufficient to normalize serum calcium concentration (10). It is possible that in the chronically hypocalcemic vitamin D-deficient state, PTH-induced stimulation of 1α-hydroxylase is limited because chronic, severe hyperparathyroidism has down-regulated the receptor sites for parathyroid hormone (49, 50), or because enzyme activity is already maximal (24, 48). But it is also possible that in frank vitamin D deficiency, stimulation of 1α-hydroxylase by PTH may be limited by the absence of 1,25-$(OH)_2D$: In rats fed a vitamin D-deficient, low calcium diet, provision of increasing amounts of vitamin D which induced progressive increases in the circulating levels of 1,25-$(OH)_2D$ was attended by progressive increases in 1α-hydroxylase activity, despite a progressively less severe hypocalcemia and presumably a less severe hyperparathyroidism (21). Whatever the mechanism by which 1α-hydroxylase became unresponsive to stimulation by PTH in the vitamin D-deprived chicks that had become hypocalcemic, the progressively increasing responsiveness to PTH demonstrated during continued but less severe deprivation of vitamin D could not have been predicted from the results obtained after hypocalcemia had supervened. Thus, the current study again demonstrates that observations made during abnormal metabolic circumstances may bear little on the physiologic regulation of 1α-hydroxylase under normal or near-normal metabolic circumstances.

Acknowledgments

We thank Dr. Bernard P. Halloran for measuring plasma concentrations of 1,25-$(OH)_2D$. We thank Ms. Bernadine Serena and Ms. Darlene De Manicor for technical assistance, and Ms. Alcyne Wong for help in preparation of this manuscript.

This work was supported by grants from the National Institutes of Health: National Institute of Arthritis, Metabolism and Digestive Diseases, AM 21354, the Division of Research Resources, General Clinical Research Center, RR-00079, and by generous gifts from the Church and Dwight Corp., and the Emil Mosbacher, Jr. Foundation.

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