The Role of 1α,25-Dihydroxyvitamin D in the Mediation of Intestinal Hyperabsorption of Calcium in Primary Hyperparathyroidism and Absorptive Hypercalciuria

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ABSTRACT The cause for the intestinal hyperabsorption of calcium (Ca) in various forms of hypercalciurias was explored by a careful measurement of plasma 1α,25-dihydroxycholecalciferol [1α,25-$(OH)_2$D] and by an assessment of intestinal Ca absorption and of parathyroid function. In 18 cases of primary hyperparathyroidism (PHPT), the mean plasma concentration of 1α,25-$(OH)_2$D was significantly increased (4.9±2.2 SD ng/dl vs. 3.4±0.9 ng/dl for the control group), and was significantly correlated with fractional Ca absorption (α) ($r = 0.80$, $P < 0.001$). Plasma 1α,25-$(OH)_2$D was also correlated with urinary Ca ($P < 0.05$), but not with serum Ca or phosphorus (P), P clearance, urinary cyclic AMP, or serum immunoreactive parathyroid hormone. In 21 cases of absorptive hypercalciuria (AH), plasma 1α,25-$(OH)_2$D was elevated in one-third of cases, and the mean value of 4.5±1.1 ng/dl was significantly higher than that of the control group ($P < 0.01$). Since relative hypoparathyroidism may be present, the normal absolute value of plasma 1α,25-$(OH)_2$D, found in two-thirds of cases of AH, may be considered to be inappropriately high. Moreover, in the majority of cases of AH, the data points relating plasma 1α,25-$(OH)_2$D and α fell within 95% confidence limits of values found in non-AH groups (including PHPT). The results suggest that the intestinal hyperabsorption of Ca in PHPT and AH may be vitamin D dependent. However, the disturbance in vitamin D metabolism may not be the sole cause for the high Ca absorption in AH, since in some patients with AH, the intestinal Ca absorption appears to be inappropriately high for the level of plasma 1α-25-$(OH)_2$D.

INTRODUCTION

The intestinal absorption of calcium (Ca) is frequently elevated in Ca urolithiasis (1–4). In resorptive hypercalciuria of primary hyperparathyroidism (PHPT)[1] and in renal hypercalciuria (RH), the intestinal absorption of Ca is often high (4). In absorptive hypercalciuria (AH), the hyperabsorptive state is invariably present and probably accounts for the hypercalciuria (4).

The exact cause for the intestinal hyperabsorption of Ca remains obscure. However, an important etiologic role for vitamin D has been suggested (5). In PHPT and RH, the hyperabsorptive state may be associated with the excessive secretion of parathyroid hormone (PTH), since it may be ameliorated by parathyroidectomy (6) and produced by exogenous administration of the hormone (7). This action of PTH may be mediated through 1α,25-dihydroxycholecalciferol [1α,25-$(OH)_2$D], since the PTH administration has been shown to stimulate the synthesis of this active metabolite of vitamin D (8). In AH, the plasma concentration of 1α,25-$(OH)_2$D has been reported to be increased (9), even though parathyroid function is normal or suppressed (4).

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[1] Abbreviations used in this paper: α, fractional calcium absorption; 1α,25-$(OH)_2$D, 1α,25-dihydroxycholecalciferol; AH, absorptive hypercalciuria; cAMP, cyclic AMP; Cr, creatinine; iPTH, immunoreactive parathyroid hormone; NN, normocalciuric nephrolithiasis; PHPT, primary hyperparathyroidism; PTH, parathyroid hormone; RH, renal hypercalciuria.

These circumstances prompted us to examine in detail the pathogenetic role of plasma $1\alpha,25\text{-}(OH)_2D$ concentration in the mediation of intestinal hyperabsorption of Ca in well-defined cases with PHPT, RH, and with AH.

METHODS

Study protocol. A slight modification of a previously reported protocol was used. This protocol allowed a simultaneous measurement of intestinal Ca absorption, parathyroid function, and of circulating concentration of $1\alpha,25\text{-}(OH)_2D$ during a constant dietary regimen. Patients were maintained on a constant liquid synthetic diet (Calcitest, The Doyle Pharmaceutical Co., Minneapolis, Minn.) with a daily composition of 400 mg Ca, 800 mg phosphorus (P), and 100 meq sodium (Na) for 3 days. Urine specimens were collected in 24-h pools during these 3 days and analyzed for Ca, P, cyclic AMP (cAMP), and creatinine (Cr). On days 2–4, venous blood samples were obtained without stasis, during fasting state before breakfast each day, for Ca, P, Cr, and proteins. Since determinations varied by less than 5% in the same patient, mean values for serum Ca and P are presented here. On day 3, venous blood sample was obtained similarly and assayed for immunoreactive PTH (iPTH) and for $1\alpha,25\text{-}(OH)_2D$. On the same day, fractional Ca absorption (α) was measured by recovery of ^{47}Ca in the feces after oral administration of the isotope (4). Food was withheld for 4 h after the oral administration of ^{47}Ca; thereafter, the patients were maintained on the liquid synthetic diet. Stool was collected until the appearance of carmine, which was given 24 h after the isotope administration.

Clinical data. This report considers study in 46 patients and 11 control subjects at the General Clinical Research Center. The protocol used was approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas, Tex. Informed consents were obtained before study.

18 patients with PHPT, 3 with RH, 21 with AH, 4 with normocalciuric nephrolithiasis (NN), and 11 normal volunteers (control group) participated in the study. These diagnoses were reached after an extensive evaluation of parathyroid function and Ca metabolism (see study protocol) (4, 10, 11). Pertinent laboratory data are summarized in Table I. In PHPT, there were significant increases in serum Ca, iPTH, urinary cAMP, intestinal Ca absorption (α), and urinary Ca. Subsequent to this study, 15 cases underwent parathyroid exploration; the diagnosis of PHPT was confirmed by the demonstration of parathyroid adenoma in 14 and of parathyroid hyperplasia in 1. The three cases of RH had increased renal excretion of Ca (>200 mg/day on a diet containing daily 400 mg Ca and 100 meq Na); fasting urinary Ca after an overnight fast (not shown in table) was elevated (10). Despite normal serum Ca, parathyroid function was stimulated as shown by high values for urinary cAMP and (or) serum iPTH. The characteristic features of AH were: normocalcemia, hypercalciuria, intestinal hyperabsorption of Ca (α of >0.61 [4]), and normal or suppressed values for serum iPTH and urinary cAMP. In NN (11), features were essentially the same as in the control group, although urinary Ca was high normal and urinary cAMP significantly reduced. All patients with RH, AH, and NN presented with a history of recurrent passage of Ca-containing renal stones. The control group consisted of normal volunteers without a history of renal stones or bone disease. None of the patients or control subjects had received thiazides, anticonvulsants, steroids, supplemental phosphates, or vitamin D for 4 mo before the study.

Analytical procedures. Calcium was determined by atomic absorption spectrophotometry, and P by the method of Fiske and SubbaRow (12). Creatinine was measured in an AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). Urinary cAMP was analyzed by the protein binding assay of Gilman (13). Serum iPTH was measured by a modification (14) of a previously described radioimmunoassay procedure (15, 16). This assay utilizes an antisera (chicken) directed against the carboxy-terminal region of bovine PTH, bovine PTH as tracer, and purified human PTH as standard. The assay can detect in essentially all normal subjects basal hormone concentrations which range from 0.20 to 0.80 pg/ml of human PTH, but there is approximately 10% overlap with hyperparathyroidism subjects; intra- and interassay variations are approximately 10% and 20%, respectively. Calcium and EDTA infusion produce a rapid decrease and increase, respectively, in plasma hormone concentrations (15, 16).

Plasma $1\alpha,25\text{-}(OH)_2D$ was measured by radioreceptor assay of Brumbaugh et al. (17, 18). With this method, values for the metabolite have been shown to approximate results by bioassay (19) and to be decreased in patients with renal failure or hypoparathyroidism (20). The mean value for 11 control subjects in Dallas was 3.4 ± 0.9 SD ng/dl, which was not significantly different from that of 3.4 ± 0.7 ng/dl in 50 control subjects at Tucson, Ariz. The mean $+2$ SD of control values in Tucson was used to define the upper limits of normal in this study. There was no significant difference in values between male and female control subjects. Each sample was assayed in triplicate; results were acceptable only if the replicate values agreed within 10%. In two control subjects, plasma $1\alpha,25\text{-}(OH)_2D$ was measured on four different occasions over a period of 8 mo. It ranged from 4.3 to 5.6 ng/dl in one subject, and from 2.8 to 3.3 ng/dl in the other.

Renal clearance of P and Cr were calculated from 24-h values for urinary P and Cr and fasting serum P and Cr, respectively.

RESULTS

Plasma $1\alpha,25\text{-}(OH)_2D$. In PHPT, plasma concentration of $1\alpha,25\text{-}(OH)_2D$ was elevated (greater than 4.8 ng/dl) in 7 of 18 cases (Fig. 1). The mean value of 4.9 ± 2.2 SD ng/dl was significantly higher than that for the control group ($P < 0.01$) (Table I). There was also a significant correlation between $1\alpha,25\text{-}(OH)_2D$ and α in PHPT ($r = 0.80, P < 0.001$). There was also a significant correlation between $1\alpha,25\text{-}(OH)_2D$ and α in the combined group of PHPT, RH, NN, and control subjects ($r = 0.83, P < 0.001$) (Fig. 1). The slopes and intercepts of the two lines of regression did not differ significantly. The values for $1\alpha,25\text{-}(OH)_2D$ in PHPT also correlated significantly with urinary Ca ($P < 0.05$), but now with serum Ca or P, urinary P, phosphorus clearance, urinary cAMP, or serum iPTH.

In AH, plasma $1\alpha,25\text{-}(OH)_2D$ was increased (>4.8 ng/dl) in 7 of 21 cases (Fig. 2), and the mean value was significantly greater than that of the control group (Table I). Most of the values in AH (14 of 21) were within the 95% confidence limits for the values in non-AH groups (PHPT, RH, NN, and control) (Fig. 2).
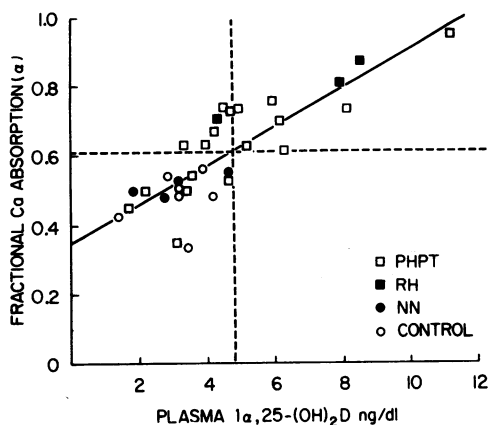
FIGURE 1 Dependence of fractional Ca absorption (α) on plasma concentration of $1\alpha,25\text{-}(OH)_2D$ in PHPT and in RH. The horizontal dashed line indicates upper range of normal for α (4) and the vertical dashed line represents the upper limit of normal for plasma $1\alpha,25\text{-}(OH)_2D$. The upper range of normal for $1\alpha,25\text{-}(OH)_2D$ is given by mean+SD for values in control subjects in Tucson, Ariz. The diagonal line represents regression for the combined group of PHPT, RH, NN, and control.

When the values in AH were added to those of other groups, the equation obtained was: $\alpha = 0.435 \pm 0.048$ $(1\alpha,25\text{-}(OH)_2D)$, whereas the equation for the combined group of PHPT + RH + NN + control (without AH) was $\alpha = 0.350 \pm 0.056$ $(1\alpha,25\text{-}(OH)_2D)$. By the

Student's t-test, there was no significant difference between slopes of the two equations, but a significant difference in intercepts $(P < 0.01)$.

Plasma $1\alpha,25\text{-}(OH)_2D$ was not correlated with serum or urinary Ca or P, phosphorus clearance, urinary cAMP, or with serum iPTH. In this group of patients with AH, serum P, urinary P, and P clearance did not differ significantly from those of the control group.

DISCUSSION

Hypercalciuria associated with Ca urolithiasis may be classified into three main types; resorptive hypercalciuria of PHPT, RH, and AH (2, 4, 10, 21). The primary cause for the hypercalciuria in the three conditions has been attributed to the excessive skeletal resorption, an impairment in the renal tubular reabsorption of Ca, or an intestinal hyperabsorption of Ca, respectively (4). The absorptive and renal hypercalciurias probably constitute the major variants of "idiopathic" hypercalciuria. An important differentiating feature is the parathyroid stimulation in PHPT and RH (21), and normal or suppressed state in AH (4, 10). A feature shared by the three forms of hypercalciuria is the intestinal hyperabsorption of Ca, which is characteristic of AH and frequently found in PHPT and RH (4, 5, 10). Because of the well-known vitamin D action on intestinal Ca transport, an important pathogenetic role for vitamin D has been implicated.

TABLE I
Clinical and Laboratory Presentations in Five Groups

	PHPT	RH	AH	NN	Control group
Patients, n	18	3	21	4	11
Age, yr	50±12	43±9	40±13	34±13	35±9
Male/female, n	7/11	2/1	17/4	4/0	6/5
Serum Ca, mg/dl	11.0±0.5*	9.6±0.1	9.4±0.3	9.6±0.1	9.7±0.5
Serum P, mg/dl	3.12±0.58‡	3.60±0.10	3.70±0.50	3.72±0.41	3.74±0.70
Urinary Ca, mg/day	239±145*	301±104§	208±43*	157±48‡	100±37
Urinary P, mg/day	631±162	724±147	717±140	643±149	698±153
P clearance, ml/min	14.1±4.1	14.0±3.2	13.5±3.4	12.0±3.6	13.0± 5.7
Cr clearance, ml/min	88±27‡	112±36	107±27	95±23	113±27
Urinary cAMP μmol/g Cr	7.38±1.79*	5.68±0.23§	3.55±1.11*	3.75±0.23§	4.70±0.41
Serum iPTH, ng/ml	5.64±4.72	—	0.54±0.21	0.63±0.04	—
Fractional Ca absorption, α	0.64±0.14§	0.80±0.09*	0.73±0.07*	0.51±0.03	0.48±0.08
Plasma 1α,25-(OH)₂D, ng/dl	4.9±2.2§	6.9±2.3‡	4.5±1.1§	3.1±1.2	3.4±0.9
Fasting Urinary Ca, mg/mg Cr	0.17±0.10*	0.17±0.05*	0.07±0.02	0.05±0.02	0.05±0.03

Sera from RH and control group were inadvertantly not assayed for iPTH. The control value for this assay (14–16) is reported to be less than 800 pg/ml. With another assay (4), using CH 14M antiserum of Arnaud (22), serum iPTH was elevated in all three cases of RH (4). In the control group, α was measured in only seven cases. Values are presented as mean±SD. Significance from values in the control group, obtained by Student's t test, is given as follows.
* $P < 0.001$.
‡ $P < 0.05$.
§ $P < 0.01$.

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The present study indicates that a disturbance in vitamin D metabolism may be present in certain forms of hypercalciurias. In states of hyperparathyroidism (PHPT and RH), this abnormality may account for the intestinal hyperabsorption of Ca. Thus, plasma concentration of $1\alpha,25$-$(OH)_2D$ was significantly increased and positively correlated with intestinal Ca absorption. In a preliminary study, treatment of control subjects with crude parathyroid extract (Eli Lilly and Co., Indianapolis, Ind.) significantly raised α commensurate with a rise in plasma concentration of $1\alpha,25$-$(OH)_2D$ (22). However, the exact stimulus for the synthesis of $1\alpha,25$-$(OH)_2D$ in hyperparathyroidism is not clear, since the plasma concentration for the vitamin D metabolite was not correlated with serum Ca or P, immunoreactive PTH, or urinary cAMP. It is also not known why only some of the patients with PHPT had increased circulating concentration of $1\alpha,-25$-$(OH)_2D$. The results could be explained if the synthesis of $1\alpha,25$-$(OH)_2D$ depend on direct or indirect effects of PTH at the level of the renal mitochondria, and not on circulating concentrations of PTH, Ca, or of P.

In AH, a disturbance in vitamin D metabolism may also be etiologically important in the mediation of intestinal hyperabsorption of Ca. First, plasma concentration of $1\alpha,25$-$(OH)_2D$ was clearly increased in one-third of cases of AH. Since relative hypoparathyroidism may be present in AH (4), the normal absolute values of the vitamin D metabolite, found in the majority of cases of AH, may be considered to be inappropriately high. Thus, there may be an increased synthesis of $1\alpha,25$-$(OH)_2D$ in AH. Secondly, in the majority of cases of AH, the data points relating plasma $1\alpha,25$-$(OH)_2D$ and α fall within the 95% confidence limits of values in non-AH groups. Moreover, the slopes of the regression line for α were not significantly altered when the values in AH were added to those in other groups. The results suggest that the stimulation of $1\alpha,25$-$(OH)_2D$ production may be a major determinant of the increased intestinal Ca absorption in AH.

However, a disturbance in vitamin D metabolism may not be the sole cause for the high intestinal absorption of Ca in AH. In 7 of 21 cases, the data points relating plasma $1\alpha,25$-$(OH)_2D$ and α were outside the 95% confidence limits of values in non-AH groups. The intercept of the regression line for α was significantly higher when values in AH were added to other groups.

The results indicate that in some patients with AH, particularly those with normal absolute values of plasma $1\alpha,25$-$(OH)_2D$, the intestinal Ca absorption may be inappropriately high for the level of $1\alpha,25$-$(OH)_2D$. It is interesting to speculate that a prolonged stimulation of $1\alpha,25$-$(OH)_2D$, consistent with the chronic nature of AH, may have caused "hypertrophy" of

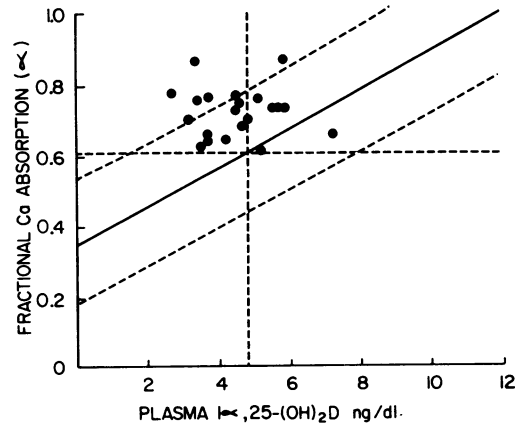

FIGURE 2 Relationship between fractional Ca absorption and plasma concentration of $1\alpha,25$-$(OH)_2D$ in resorptive hypercalciuria. Each point represents a study in separate patients. The solid diagonal line indicates the regression line and the dashed diagonal curves are the 95% confidence limits for values in PHPT, RH, NN, and control.

intestinal cells concerned with Ca absorption, analogous to the effect of high gastrin production on parietal cell population in stomach (23). Such a hypertrophy could account for the high α relative to plasma concentration of $1\alpha,25$-$(OH)_2D$ in AH. However, it is also possible that AH may consist of a group in which intestinal hyperabsorption of Ca is vitamin D dependent, and another group in which high Ca absorption is vitamin D independent.

The cause for the apparent increased synthesis of $1\alpha,25$-$(OH)_2D$ in AH is not known. Shen et al. (9) also found this increase and attributed it to hypophosphatemia, resulting from an impaired renal tubular reabsorption of P. The importance of hypophosphatemia in the regulation of the synthesis of $1\alpha,25$-$(OH)_2D$ has been stressed previously (24). However, in this study in AH, plasma concentration of $1\alpha,25$-$(OH)_2D$ was not significantly correlated with serum P. Furthermore, serum P in AH was usually normal in this, as well as in a previous study (4), when it was determined under controlled conditions of diet and fast. Nevertheless, in those with hypophosphatemic AH, the operation of P-dependent synthesis of the vitamin D metabolite is theoretically possible.

ACKNOWLEDGMENTS

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