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Phillip W. Lambert

Paula H. Stern

Richard C. Avioli

Newton C. Brackett

Russell T. Turner

See next page for additional authors

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Authors

Phillip W. Lambert, Paula H. Stern, Richard C. Avioli, Newton C. Brackett, Russell T. Turner, Anne Greene, Irene Y. Fu, and Norman H. Bell

Evidence for Extrarenal Production of $1\alpha,25$ -Dihydroxyvitamin D in Man

PHILLIP W. LAMBERT, PAULA H. STERN, RICHARD C. AVIOLI, NEWTON C. BRACKETT, RUSSELL T. TURNER, ANNE GREENE, IRENE Y. FU, and NORMAN H. BELL, *Veterans Administration Medical Center and Department of Medicine, Case Western Reserve University Medical School, Cleveland, Ohio 44106; Department of Pharmacology, Northwestern University Dental and Medical Schools, Chicago, Illinois 60611; Veterans Administration Medical Center and Departments of Medicine and Pharmacology, Medical University of South Carolina, Charleston, South Carolina 29403*

ABSTRACT Recent studies provide evidence for extrarenal production of $1\alpha,25$ -dihydroxyvitamin D [$1\alpha,25(\text{OH})_2\text{D}$]. To investigate this possibility, serum vitamin D, 25-hydroxyvitamin D (25-OHD), 24,25-dihydroxyvitamin D [$24,25(\text{OH})_2\text{D}$], and $1\alpha,25(\text{OH})_2\text{D}$ were measured in eight adult anephric subjects. All were undergoing hemodialysis and three of them were receiving vitamin D, 50,000 or 100,000 U/d. Serum vitamin D was elevated in two of the patients given vitamin D and was abnormally low in the others. Mean serum 25-OHD was increased in patients given vitamin D (94.0 ± 7.6 ng/ml) and was normal in the others (16.4 ± 0.9 ng/ml, $P < 0.001$). Mean serum $24,25(\text{OH})_2\text{D}$ was normal in patients given vitamin D (1.38 ± 0.27 ng/ml) and was low in the others (0.25 ± 0.08 ng/ml, $P < 0.001$). Serum $24,25(\text{OH})_2\text{D}$ correlated significantly with serum 25-OHD ($r = 0.848$, $P < 0.01$). Mean serum $1\alpha,25(\text{OH})_2\text{D}$ determined by receptor assay was 5.8 ± 1.9 pg/ml in patients who were not given vitamin D and was 14.1 ± 0.6 in those who were given vitamin D ($P < 0.001$). Serum $1\alpha,25(\text{OH})_2\text{D}$ correlated significantly with serum 25-OHD ($r = 0.911$, $P < 0.01$). Mean serum $1\alpha,25(\text{OH})_2\text{D}$, measured by bioassay, was 8.3 ± 1.9 pg/ml in patients who were not given vitamin D and was 15.9 ± 2.4 pg/ml in those who were given vitamin D ($P < 0.05$). There was a significant correlation between the values for serum $1\alpha,25(\text{OH})_2\text{D}$ obtained with the two methods ($r = 0.728$, $P < 0.01$). The results (a) provide evidence in man for extrarenal production of both $24,25(\text{OH})_2\text{D}$ and, by two independent assays, of $1\alpha,25(\text{OH})_2\text{D}$, and (b) indicate that serum

values of the two dihydroxy metabolites of vitamin D in anephric subjects vary with the serum concentration of the precursor 25-OHD.

INTRODUCTION

It is widely accepted that $1\alpha,25$ -dihydroxyvitamin D [$1\alpha,25(\text{OH})_2\text{D}$]¹ is produced only by the kidneys. A number of laboratories, including our own, reported undetectable values for serum $1\alpha,25(\text{OH})_2\text{D}$ in anephric individuals by receptor assay (1-5), radioimmunoassay (6, 7), and bioassay (8). In one study, low but detectable values were found in three patients by receptor assay. In this study, it was not clear whether the findings represented extrarenal production, intake of exogenous $1,25(\text{OH})_2\text{D}$, or an artefact (9). Recent evidence suggests extrarenal synthesis of the metabolite: conversion of [³H]25-hydroxyvitamin D₃ (25-OHD) to [³H] $1\alpha,25(\text{OH})_2\text{D}_3$ by cultured human bone cells (10) and by cultured chick calvarial cells (11) was demonstrated. The structure of the $1\alpha,25(\text{OH})_2\text{D}_3$ biologically synthesized by the chick calvarial cells was verified by mass spectroscopy (Dr. Turner, unpublished observations). Also, a patient with sarcoid was reported in whom hypercalcemia, increased circulating $1\alpha,25(\text{OH})_2\text{D}$, and suppressed serum immunoreactive parathyroid hormone (PTH) were present after bilateral nephrectomy (12). The serum calcium varied directly with the serum $1\alpha,25(\text{OH})_2\text{D}$. Hypercalcemia and the elevated serum $1\alpha,25(\text{OH})_2\text{D}$ in this

Address reprint requests to Dr. Bell, Veterans Administration Medical Center, Charleston, SC.

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¹ Abbreviations used in this paper: $1,25(\text{OH})_2\text{D}$, 1,25-dihydroxyvitamin D; $24,25(\text{OH})_2\text{D}$, 24,25-dihydroxyvitamin D; 25-OHD, 25-hydroxyvitamin D; PTH, parathyroid hormone.

patient returned to normal upon treatment with glucocorticoids.

In view of these findings, we measured serum $1\alpha,25(\text{OH})_2\text{D}$ in a group of anephric individuals. Evidence obtained with receptor assay (5) and bioassay (8) is presented, indicating that low but detectable serum values of the metabolite are regularly present in the absence of the kidneys. Further, the values are higher in patients on treatment with vitamin D_2 , and there is a positive correlation between serum 25-OHD and serum $1\alpha,25(\text{OH})_2\text{D}$ in anephric subjects.

METHODS

Eight anephric patients, two men and six women, were studied. They ranged in age from 33 to 48 yr. All of them were on treatment with hemodialysis. Three of them were studied during admission to either the Medical University Hospital or the Charleston Veterans Administration Medical Center. The other five were studied while they were treated as outpatients in the Dialysis Clinic. At the time of evaluation, none of them had received blood transfusions for at least 2 d. None were on treatment with 1α -hydroxyvitamin D_3 , $1\alpha,25(\text{OH})_2\text{D}_3$, or dihydrotachysterol.

Blood samples were obtained in the fasting state for determination of serum calcium, phosphorus, creatinine, immunoreactive PTH, vitamin D, 25-OHD, 24,25-dihydroxyvitamin D [$24,25(\text{OH})_2\text{D}$], and $1\alpha,25(\text{OH})_2\text{D}$. Serum calcium (13), phosphorus (14), and creatinine (15) were determined by automated methods. Serum immunoreactive PTH was measured by radioimmunoassay with antiserum from chicken 77125 at a final concentration of 1:10,000 (16). Normal values are 0.28 ± 0.12 ng/ml (mean \pm SD) in a group of 85 normal adults between the ages of 21 and 49 yr (16). Serum vitamin D, 25-OHD, and $24,25(\text{OH})_2\text{D}$ were assayed after extraction and chromatography by the protein binding method with normal rat serum (5, 17). Normal values are 2.6 ± 0.8 ng/ml (mean \pm SD, $n = 21$) for vitamin D, 27.7 ± 6.4 ng/ml ($n = 95$) for 25-OHD, and 1.4 ± 0.6 ng/ml ($n = 85$) for $24,25(\text{OH})_2\text{D}$ (5). Serum $1\alpha,25(\text{OH})_2\text{D}$ was measured by assay with the cytosol receptor from chick intestinal mucosa after extraction and chromatography (5, 17). Normal values are 34.2 ± 7.4

pg/ml ($n = 65$). Serum $1\alpha,25(\text{OH})_2\text{D}$ was also determined by bioassay (8). Normal values are 32.0 ± 11.3 pg/ml.

Student's t test was used to determine the significance of differences of unpaired samples. Correlation coefficient and Student's t test were done with a calculator (model 9815 A, Hewlett-Packard Co., Palo Alto, Calif.).

RESULTS

Clinical findings in the patients are summarized in Table I. All were undergoing treatment with chronic hemodialysis and three of them were being treated with vitamin D_2 . Each had marked elevation of serum creatinine. Patient B had hypocalcemia (with a normal serum immunoreactive PTH) as a result of parathyroidectomy despite a daily intake of 100,000 U of vitamin D_2 . Two patients were hypercalcemic. In patient A serum immunoreactive PTH was normal, and in patient H it was increased. Serum immunoreactive PTH was abnormally elevated in each of the other patients, all of whom had a normal serum calcium. Serum phosphorus was normal in four patients as a result of treatment with aluminum gels and was elevated in each of the other patients.

The values for circulating vitamin D and its metabolites are summarized in Table II. Serum vitamin D was elevated in two of the three patients on treatment with vitamin D and was abnormally low in the third individual. It was abnormally reduced in each of the other five patients. Mean serum 25-OHD was increased in patients given vitamin D (94.0 ± 7.6 ng/ml, ± 1 SE) and was normal in those who were not given the vitamin (16.4 ± 0.9 ng/ml, $P < 0.001$). Mean serum $24,25(\text{OH})_2\text{D}$ was normal in patients taking vitamin D (1.38 ± 0.27 ng/ml) and was significantly reduced in those who were not given the vitamin (0.25 ± 0.08 ng/ml, $P < 0.001$). There was a positive correlation between serum 25-OHD and serum $24,25(\text{OH})_2\text{D}$ ($r = 0.848$, $P < 0.01$). Mean serum $1\alpha,25(\text{OH})_2\text{D}_2$, measured

TABLE I
Clinical Findings in Anephric Patients

Patient	Age	Sex	Serum				Treatment		
			Ca	P	Creat	PTH	Hemodialysis	Parathyroidectomy	Vitamin D_2
	yr		mg/dl	mg/dl	mg/dl	ng/ml			U/d
A	48	F	11.3	5.2	11.8	0.74	+	-	100,000
B	46	F	7.4	4.2	12.0	0.44	+	+	100,000
C	34	F	9.4	6.4	15.5	1.89	+	-	-
D	48	M	9.3	3.1	9.4	1.65	+	-	-
E	45	F	8.4	8.1	17.2	1.38	+	-	50,000
F	34	F	10.0	6.2	15.0	2.75	+	-	-
G	48	M	8.8	3.3	11.6	1.30	+	-	-
H	33	F	11.6	4.1	13.5	2.25	+	-	-

TABLE II
Serum Vitamin D, 25-OHD, 24,25(OH)₂D, and 1α,25(OH)₂D
in Anephric Patients

Patient	Serum				
	Vitamin D	25-OHD	24,25(OH) ₂ D	1α,25(OH) ₂ D*	
				a	b
	ng/ml	ng/ml	ng/ml	pg/ml	
A †	107.0	89.5	0.56	14.4	14.9
	37.5	109.5	1.71	13.1	15.9
B †	0.41	93.8	1.10	14.7	11.4
	0.32	105.9	1.90	14.0	13.3
C	0.81	16.9	0.06	3.2	3.5
	0.05	4.2	0.16	3.9	4.9
D	0.05	16.4	0.03	—	7.1
	0.09	17.4	0.29	6.4	15.4
E †	10.8	71.2	1.61	14.1	23.8
F	0.18	16.7	0.22	7.7	10.3
G	0.06	16.4	0.46	7.9	—
H	0.20	14.8	0.53	5.6	8.8
Normal values:	2.6±0.8	27.7±6.4	1.4±0.6	34.2±7.4	32.0±11.3

* a is by receptor assay and b is by bioassay. There was a significant correlation between the values for the two methods ($r = 0.728$, $P < 0.01$).

† On treatment with vitamin D₂ (see Table I).

The interval between samples in patients A–D was 6 mo or greater. Normal values are given as mean±SD.

by receptor assay, was significantly higher in patients who were given vitamin D (14.1 ± 0.6 pg/ml) than in those who were not given the vitamin (5.8 ± 1.9 pg/ml, $P < 0.001$). There was a positive correlation between serum 25-OHD and serum 1α,25(OH)₂D ($r = 0.911$, $P < 0.01$). Mean serum 1α,25(OH)₂D, measured by bioassay, was similarly higher in patients given vitamin D (15.9 ± 2.4 pg/ml) than in those who were not given the vitamin (8.3 ± 1.9 pg/ml, $P < 0.05$). There was a significant correlation between the serum values for 1α,25(OH)₂D with the two methods ($r = 0.728$, $P < 0.01$).

DISCUSSION

Our findings indicate that 24,25(OH)₂D and 1α,25(OH)₂D are regularly present in the sera of anephric patients. As regards 24,25(OH)₂D, previous results in anephric subjects were conflicting. Values were reported as being either low (5, 18–20) or undetectable (20, 21). Our results showing increases in serum

24,25(OH)₂D during treatment with vitamin D are consistent with those recently reported in anephric pigs (20). The failure to demonstrate detectable serum values in previous studies may be due to differences in methods, including sensitivity and accuracy of the binding assays and recovery after extraction and purification of serum samples (5).

Previous unsuccessful attempts to demonstrate 1α,25(OH)₂D in sera from anephric individuals may have occurred because the quantity of serum used was inadequate. This was clearly the case in our previously reported studies (5, 8). These and our present results were obtained with two quite independent and highly sensitive assays: one uses the displacement of [³H]-1α,25(OH)₂D₃ from chick intestinal cytosol receptor (5, 17) and the second uses the release of ⁴⁵Ca from long bones of fetal rats in tissue culture (8). With both methods, the putative 1α,25(OH)₂D found in the present report was purified by extraction and purification by high pressure liquid chromatography. It is unlikely that another metabolite with the same chromatographic and biologic properties of 1α,25(OH)₂D was isolated. In previous studies, these two assay systems showed a remarkable degree of correlation in their responses to a wide range of analogues of vitamin D (22). Our findings, therefore, provide strong evidence that there are sites other than the kidney for the production of 1α,25(OH)₂D in man.

As noted already, evidence for extrarenal production of 1α,25(OH)₂D was reported in an anephric patient with sarcoidosis (12). Synthesis of the metabolite was demonstrated in cultured human bone cells (10), cultured chick calvarial cells (11), human decidua (23), and rat placenta (24).

Under normal circumstances, circulating serum 1α,25(OH)₂D is closely regulated by serum PTH so that there is no correlation between the serum 25-OHD and serum 1α,25(OH)₂D (17). However, in the absence of PTH (that is, in patients with hypoparathyroidism), there is positive correlation between serum 25-OHD and serum 1α,25(OH)₂D (25). In our studies there was a similar positive correlation in anephric patients, most of whom had secondary hyperparathyroidism but one of whom had had a parathyroidectomy. Also, a positive correlation between serum 25-OHD₃ and serum 1α,25(OH)₂D₃ was observed in children with chronic renal failure who were on treatment with 25-OHD₃ when the glomerular filtration rate was ≤ 25 ml/min (26). Further studies are indicated to characterize the metabolic regulation of human 25-OHD-1α-hydroxylases in extrarenal tissue.

All of the patients who were not receiving vitamin D exhibited low values for circulating vitamin D. Preliminary results suggest impairment in the photoproduction of the vitamin in the epidermis of patients

who are on chronic hemodialysis (27). We have no explanation for the low values in one of our patients who was on treatment with vitamin D.

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