Chronic 1,25-Dihydroxyvitamin D_3 Administration in the Rat Reduces the Serum Concentration of 25-Hydroxyvitamin D by Increasing Metabolic Clearance Rate

Bernard P. Halloran, Daniel D. Bikle, Marilyn J. Levens, Margaret E. Castro, Ruth K. Globus, and Emily Holton*
*Veterans Administration Medical Center and Departments of Medicine and Physiology, University of California, San Francisco, California 94121; *National Aeronautics and Space Administration-Ames Research Center, Biomedical Research Division, Moffett Field, California 94035*

Abstract

Administration of 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] can lower the serum concentration of 25-hydroxyvitamin (25-OH-D). To determine if 1,25$(OH)_2D_3$ lowers serum 25-OH-D by increasing clearance or reducing production, we directly measured the metabolic clearance rate (MCR) of 25-OH-D in rats chronically infused with 1,25$(OH)_2D_3$. Chronic 1,25$(OH)_2D_3$ administration (0 to 75 pmol/d) reduced, in a time- and dose-dependent fashion, the serum concentrations of 25-OH-D_3 and 24,25$(OH)_2D_3$ from 18±2 to 9±1 ng/ml and from 4.8±0.7 to 1.3±0.3 ng/ml, respectively, and increased sevenfold the in vitro conversion of 25-OH-D to 24,25$(OH)_2D_3$ by kidney homogenates. The reduction in serum 25-OH-D_3 was completely accounted for by an increase in MCR. No change in production occurred. The influence of 1,25$(OH)_2D_3$ on serum 25-OH-D_3 and 24,25$(OH)_2D_3$ was shown not to be dependent on induction of hypercalcemia. These data suggest that chronic 1,25$(OH)_2D_3$ administration lowers serum 25-OH-D by increasing the metabolic clearance of 25-OH-D_3 and not by decreasing its production.

Introduction

25-Hydroxyvitamin D (25-OH-D)[1] is produced in the liver by hydroxylation of vitamin D. 25-Hydroxyvitamin D in turn acts as substrate for production of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D], the most biologically active form of the vitamin (1, 2).

Most evidence suggests that the serum concentration of 25-OH-D is only loosely regulated and under most circumstances appears to be primarily dependent on the circulating concentration of vitamin D (3–6). Recent evidence, however, has been interpreted to suggest that 1,25$(OH)_2$D may feed back to inhibit synthesis of 25-OH-D (7–13). For example, administration of 1,25$(OH)_2$D has been shown to reduce the response of serum 25-OH-D_3 to challenge with vitamin D_3 (11), and osteoporotic patients given 1,25$(OH)_2D_3$ have been shown to have lower serum concentrations of 25-OH-D after 1,25$(OH)_2D_3$ therapy (8). Numerous other clinical observations support the fact that 1,25$(OH)_2D_3$ can reduce circulating 25-OH-D concentrations (7, 9, 12). Furthermore, it has been shown in vitro that 1,25$(OH)_2D_3$ at physiological concentrations can reduce accumulation of [^{3}H]25-OH-D_3 produced from ^{3}H-vitamin D_3 in liver homogenates and perfused livers from rachitic rats (10). Taken collectively, these data support the hypothesis that 1,25$(OH)_2$D can inhibit 25-OH-D synthesis. However, the data can equally well be explained by a 1,25$(OH)_2$D-induced increase in metabolic turnover or catabolism of 25-OH-D. To determine if administration of 1,25$(OH)_2$D in vivo lowers serum 25-OH-D by reducing production or increasing metabolic clearance, we infused rats with 1,25$(OH)_2D_3$ for 12 d and directly measured the metabolic clearance rate (MCR) of 25-OH-D_3. We also examined the influence of chronic 1,25$(OH)_2D_3$ administration on the serum concentration of 24,25-dihydroxyvitamin D [24,25$(OH)_2D_3$], a major metabolic product of 25-OH-D and on the in vitro metabolism of 25-OH-D_3, 24,25$(OH)_2D_3$, and 1,25$(OH)_2D_3$ by liver and kidney homogenates. The results indicate that chronic administration of 1,25$(OH)_2D_3$ can lower the serum concentrations of both 25-OH-D_3 and 24,25$(OH)_2D_3$, but, unlike previous in vitro reports, suggest that 1,25$(OH)_2D_3$ lowers serum 25-OH-D_3 by increasing its metabolic clearance and not by decreasing its production.

Methods

Animal protocols. Male Sprague-Dawley rats (Hilltop Laboratories, Scottsdale, PA) weighing 100–125 g were fed ad lib. either standard rat chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL, F-6) containing 4.41 U vitamin D_3/g of food, 1.2% Ca and 0.99% P, or one of three synthetic vitamin D–deficient diets supplemented with vitamin D_3 (100 U vitamin D_3/d) and containing either 0.4, 1.2, or 2.4% Ca, and 1.2% P, respectively (14). All animals were maintained on their respective diets for at least 10 d before experimentation and at the time of killing were 55 d of age in each experiment.

To examine the effect of chronic 1,25$(OH)_2D_3$ administration on the serum concentrations of 1,25$(OH)_2D_3$, 25-OH-D_3, and 24,25$(OH)_2D_3$, Alza osmotic minipumps (model 2002, Alza Corp., Palo Alto, CA) were implanted subcutaneously on the back and the animals were continuously infused with either vehicle (1.25% ethanol in propylene glycol) or chromatographically purified 1,25$(OH)_2D_3$ (a gift from Dr. M. Uskokovic, Hoffmann-LaRoche, Nutley, NJ) at a constant rate of 25, 50 or 75 pmol/d (12 μl/d) for 3, 7, or 12 d. At the end of the infusion period, blood was collected from the dorsal aorta while the animals were under light ether anesthesia and the serum analyzed for calcium, inorganic phosphate, and the vitamin D metabolites.

The MCR of 25-OH-D_3 was measured using the constant infusion method (15, 16). Approximately 0.5 μCi of chromatographically purified 25-OH(26,27(n)-^{3}H)D_3 (158 Ci/mmol) was solubilized in propylene glycol containing 1.25% ethanol and continuously infused subcutaneously using Alza osmotic minipumps at a rate of between 30 and 60 dpm/min (8.33 nl/min) for up to 13 d. This rate of hormone infusion is equivalent to

Address reprint requests to Dr. Bernard Halloran, Veterans Administration Medical Center M/C 11, 4150 Clement Street, San Francisco, CA 94121.

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1. *Abbreviations used in this paper:* 25-OH-D, 25-hydroxyvitamin D; 1,25$(OH)_2$D, 1,25-dihydroxyvitamin D; MCR, metabolic clearance rate; PR, production rate; PTH, parathyroid hormone.

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0.035–0.07 pg/min or 0.008–0.016% of the endogenous production rate. For all routine measurements of MCR, animals were infused for 12 d. At the end of the infusion period, animals were bled from the dorsal aorta.

Accurate measurement of the MCR using the constant infusion technique requires that the serum pool of [^{3}H]25-OH-D$_3$, at the time of measuring the MCR, be in equilibrium with all other pools of [^{3}H]25-OH-D$_3$ within the animal. To determine if the serum pool of [^{3}H]25-OH-D$_3$ was at equilibrium (i.e., the concentration of [^{3}H]25-OH-D$_3$ in the serum was constant) at the time of measuring the MCR, in preliminary experiments we measured the serum concentration of [^{3}H]25-OH-D$_3$ at 2, 6, 9, and 13 d after beginning the infusion. The serum concentrations of [^{3}H]25-OH-D$_3$ ($n = 4$) at these time points were 1,300±116, 3,405±147, 3,508±556, and 3,795±310 dpm/ml, respectively. No significant differences (Tukey test) were seen in the concentrations at days 6, 9, and 13 indicating that the serum pool of [^{3}H]25-OH-D$_3$ was in fact in equilibrium at the time of measuring the MCR (i.e., day 12).

In vitro metabolism studies. To determine the effect of chronic 1,25(OH)$_2$D$_3$ administration on the in vitro metabolism of 25-OH-D$_3$, 24,25(OH)$_2$D$_3$, and 1,25(OH)$_2$D$_3$ by liver and kidney, animals were infused with either vehicle alone or 1,25(OH)$_2$D$_3$ at the rate of 75 pmol/d for 12 d and then sacrificed. After killing, livers and kidneys were perfused in vivo with 10 ml ice-cold homogenizing buffer, excised, passed through a Krebs tissue press, and homogenized using a teflon pestle in 2 vol of the following buffer: 100 mM KCl, 10 mM Tris-HCl, 10 mM NaH_2PO_4, 10 mM Na-pyruvate, 4 mM $MgSO_4$ and 10 mM EGTA with $CaCl_2$ added to a free calcium concentration of 0.01 mM at pH 6.8. 1-ml aliquots of the homogenized tissue were combined with ~0.1 μCi of either [^{3}H]25-OH-D$_3$ (85 Ci/mmol), [^{3}H]24,25(OH)$_2$D$_3$ (64 Ci/mmol) or [^{3}H]1,25(OH)$_2$D$_3$ (158 Ci/mmol) in 10 μl of ethanol, gased with oxygen, and incubated (in duplicate) in a shaking water bath at 37°C for 2 h. The incubations were stopped by adding 3 ml of a 2:1 mixture of methanol:chloroform, the samples shaken vigorously and phase separation accomplished by adding an additional 1 ml of chloroform and 0.8 ml H_2O. After centrifugation at 3,000 rpm for 5 min in a clinical bench top centrifuge, the lower (chloroform) phase was removed and the upper aqueous phase reextracted twice with 1 ml chloroform. The chloroform extracts were combined, dried under nitrogen and resolubilized in 150 μl isopropanol/hexane (10:90) in preparation for high performance liquid chromatography. The chromatographic system consisted of a Zorbax-Sil column (Dupont Instruments, Wilmington, DE) and a solvent system of isopropanol/hexane run in a concave gradient mode (setting 9, Waters 660 programmer, Waters Associates, Milford, MA) from 3:97 to 10:90 at a flow rate of 2 ml/min. Tritium in the column effluent was monitored continuously using a Flo-One radioactive flow counter (Radiomatics Instrument and Chemical Co., Tampa, FL). Because the tissue pools of endogenous 25-OH-D, 24,25(OH)$_2$D, and 1,25(OH)$_2$D can theoretically influence the rate of metabolic conversion of [^{3}H]25-OH-D, [^{3}H]24,25(OH)$_2$D and [^{3}H]1,25(OH)$_2$D to other metabolites, we also measured the kidney and liver homogenate concentrations of endogenous 25-OH-D, 24,25(OH)$_2$D, and 1,25(OH)$_2$D.

Laboratory methods. The serum, kidney, and liver concentrations of 1,25(OH)$_2$D$_3$, 25-OH-D$_3$, and 24,25(OH)$_2$D$_3$ were measured in the following manner. 2,000–4,000 dpm each of chromatographically purified 1,25(OH)$_2$ [26,27(n)-^{3}H]D$_3$ (158 Ci/mmol), 25-OH[26,27(n)-^{3}H]D$_3$ (158 Ci/mmol), and 24,25(OH) [23,24(n)-^{3}H]D$_3$ (66 Ci/mmol) (Amersham Corp., Arlington Heights, IL) in 20 μl of ethanol were added to 2 ml of serum and the serum was allowed to sit at room temperature for 10 min. Samples were then extracted twice with 3 vol of peroxide-free diethylether (J. T. Baker Chemical Corp., Phillipsburg, NJ) followed by extraction with 4 vol of dichloromethane/methanol (3:1). After mixing for 5 min, 1 additional vol of methanol was added, the samples were shaken for an additional 2 min and the methanol-aqueous layer was removed by aspiration. The remaining solution was washed with 0.1 M sodium phosphate buffer (pH 10.5) twice. The remaining organic phase was combined with the ether extracts, dried under nitrogen, applied to a Sephadex LH-20 column (0.6 × 15 cm) and eluted in hexane/chloroform/methanol (90:10:10). The 25-OH-D$_3$ fraction from LH-20 chromatography was applied to a Waters Radial Compression Separation System (Waters Associates) using an 8-mm silica radial compression column (10 μm particle size), a solvent system of isopropanol/hexane (4:96) and a flow rate of 3 ml/min. 25-OH-D$_3$ was quantitated using a competitive protein binding assay based on vitamin D–deficient rat serum as previously described (17). Inter- and intraassay coefficients of variation for this assay are 16 and 10%, respectively.

The dihydroxyvitamin D$_3$ fraction from LH-20 chromatography was applied to two μPorosil columns in series and eluted with a solvent system of isopropanol/hexane (10:90) at a flow rate of 2 ml/min. The 1,25(OH)$_2$D$_3$ fraction from this chromatography was quantitated with the chick intestinal cytosol receptor assay as described by Shepard et al. (18). Inter- and intraassay coefficients of variation for this assay are 11 and 9%, respectively. The 24,25(OH)$_2$D$_3$ fraction from the two μPorosil columns was further chromatographed on a Zorbax-Sil column with a solvent system of isopropanol/dichloromethane (2:98) and a flow rate of 2 ml/min (19). 24,25-Dihydroxyvitamin D$_3$ was quantitated using a competitive protein binding assay based on vitamin D deficient rat serum as previously described (16). Inter- and intraassay coefficients of variation for this assay are 20 and 12%, respectively.

For measurement of the MCR, the serum concentration of [^{3}H]25-OH-D$_3$ was measured in the following manner. Approximately 1.2 μg of chromatographically purified 25-OH-D$_3$ in 20 μl of ethanol was added to each 2-ml serum sample to determine recovery. Samples were extracted and chromatographed using a C-18 Sep-Pak (Waters Associates) as described by Reinhardt et al. (12) followed by chromatography on a silica Sep-Pak (Waters Associates). Samples were applied to the silica Sep-Paks in isopropanol/hexane (4:96) and eluted with 8 ml of the same solvent. The 25-OH-D fraction was then chromatographed using an 8-mm silica radial compression column (10 μm particle size), a solvent system of isopropanol/hexane (3:97) and a flow rate of 3.5 ml/min, followed by further high performance liquid chromatography using a μBondapak C-18 column, a solvent system of water/methanol (22:78) and a flow rate of 2.0 ml/min. The 25-OH-D$_3$ fractions from the μBondapack C-18 column were dried under nitrogen and the amount of ^{3}H determined by scintillation counting. Calculation of recovery of 25-OH-D$_3$ added to the serum was accomplished by comparison of the ultraviolet light absorbance maximum at 254 nm of the sample to that of 25-OH-D$_3$ standards. Recovery of 25-OH-D$_3$ averaged 51%. The intraassay coefficient of variation for measurement of [^{3}H]25-OH-D$_3$ in serum is 5.9% ($n = 7$).

To insure that the chromatographic procedures used to purify [^{3}H]25-OH-D$_3$ from serum were adequate (i.e., that the tritium label accurately reflected authentic [^{3}H]25-OH-D$_3$ and not other substances that may have become labeled during the course of the infusion) we calculated the serum concentration of [^{3}H]25-OH-D$_3$ after each step in a series of chromatographic procedures designed to sequentially improve purity of the sample. In these experiments, 1.2 μg of nonlabeled 25-OH-D$_3$ in 20 μl of ethanol was added to each sample of a group ($n = 10$) of serum samples (2.0 ml each) drawn from a pool of serum collected from four rats that had been infused with [^{3}H]25-OH-D$_3$ for 12 d. The serum samples were extracted and chromatographed as described above with the exception that after the reverse-phase chromatography step (i.e., the μBondapak column) the 25-OH-D$_3$ fractions were rechromatographed using a Zorbax-Sil column, a solvent system of isopropanol/dichloromethane (2:98) and a flow rate of 2 ml/min. After each chromatographic step (i.e., after the first straight phase, after the reverse phase, and after the second straight phase) a fraction of the sample was removed, the ^{3}H in the fraction quantitated, and from the recovery at that step, the concentration of [^{3}H]25-OH-D$_3$ calculated. The serum concentrations of [^{3}H]25-OH-D$_3$ calculated after each stage of chromatography were 3,072±237, 3,146±170 and 3,515±198 dpm/ml, respectively. No significant differences (Tukey test) in the concentrations were seen, suggesting that the first straight phase high performance liquid chromatographic system was adequate to remove any ^{3}H-labeled contaminants from the [^{3}H]25-OH-D$_3$ fraction. To insure a margin of safety, however, we choose to use the straight phase followed by reverse-phase high performance liquid chromatographic system for all routine analyses.

Serum calcium was measured using atomic absorption spectroscopy after dilution (1/50) of serum with $LaCl_3$. Serum inorganic phosphate (P_i) was measured using the method of LeBel et al. (20).

Calculations. The MCR of 25-OH-D_3, defined as the volume of blood cleared completely and irreversibly of 25-OH-D_3 per unit time (16), is assumed to be equal to that of [^{3}H]25-OH-D_3. At infusion equilibrium, the MCR is calculated according to the relationship: MCR (ml/min) = rate of infusion of [^{3}H]25-OH-D_3 (dpm/min)/serum concentration of [^{3}H]25-OH-D_3 (dpm/ml). The production rate (PR) of 25-OH-D_3 is calculated according to the relationship: PR (pg/min) = MCR (ml/min) × serum concentration of 25-OH-D_3 (pg/ml).

Data are presented as mean±SE. Statistical analysis was performed using Student's *t* test or, where appropriate, one-way analysis of variance and Dunnett's test, or two-way analysis of variance and the Tukey multiple comparison test.

Results

Chronic administration of 1,25$(OH)_2D_3$ at a dose of 25 pmol/d significantly increased serum 1,25$(OH)_2D_3$ by 19–24 pg/ml ($P < 0.05$) (Table I). Further increases in dosage produced a nearly linear increase in the serum concentration of 1,25$(OH)_2D_3$ ($r = 0.76$, $P < 0.001$). The duration of 1,25$(OH)_2D_3$ infusion (from 3–12 d) did not influence the serum 1,25$(OH)_2D_3$ levels achieved.

After 3 d of 1,25$(OH)_2D_3$ administration, no changes in serum 25-OH-D_3 were seen except at the highest dose where a small decrement was observed (21±3 ng/ml in control and 16 + 2 ng/ml in 1,25$(OH)_2D_3$ infused) (Table I). However, after 7 and 12 d, serum 25-OH-D_3 was significantly reduced ($P < 0.001$) at all three doses of 1,25$(OH)_2D_3$. After 12 d of 1,25$(OH)_2D_3$ infusion, at a dose of 25 pmol/d, serum 25-OH-D_3 fell from a control level of 18±2 ng/ml to 12±ng/ml ($P < 0.001$). Increasing the 1,25$(OH)_2D_3$ dose to 50 pmol/d further reduced serum 25-OH-D_3 to 9±1 ng/ml. However, as the 1,25$(OH)_2D_3$ dose was increased to 75 pmol/d, no further reduction in serum 25-OH-D_3 occurred. The relationship between the serum concentrations of 1,25$(OH)_2D_3$ and 25-OH-D_3 is shown in Fig. 1. As the serum concentration of 1,25$(OH)_2D_3$ was increased to ~150 pg/ml, there was a more or less linear decrease in the serum level of 25-OH-D_3. However, for higher serum concentrations of 1,25$(OH)_2D_3$ (up to 225 pg/ml), no further reduction in serum 25-OH-D_3 was observed.

After 7 and 12 d of constant 1,25$(OH)_2D_3$ infusion the serum concentration of 24,25$(OH)_2D_3$ was also significantly reduced ($P < 0.001$) (Table I). However, unlike the serum concentration of 25-OH-D_3, a higher dose of 1,25$(OH)_2D_3$ (viz. 50 pmol/d) was required to elicit a fall in serum 24,25$(OH)_2D_3$. After 12 d of 1,25$(OH)_2D_3$ infusion, serum 24,25$(OH)_2D_3$ was virtually identical in the 0- and 25-pmol/d dose groups. At a dose of 50 pmol/d, serum 24,25$(OH)_2D_3$ fell abruptly from 4.8±0.7 ng/ml (0 dose) to 1.8±0.1 ng/ml ($P < 0.001$). Increasing the 1,25$(OH)_2D_3$ dose to 75 pmol/d reduced serum 24,25$(OH)_2D_3$ to 1.3±0.3 ng/ml, a level not significantly different from the 50-pmol/d dose group. The relationship between the serum concentrations of 1,25$(OH)_2D_3$ and 24,25$(OH)_2D_3$ is shown in Fig. 2. As the serum concentration of 1,25$(OH)_2D_3$ was increased to ~130 pg/ml, there was little change in circulating 24,25$(OH)_2D_3$. However, as serum 1,25$(OH)_2D_3$ was further increased to 150 pg/ml there was an abrupt fall in circulating 24,25$(OH)_2D_3$ from roughly 4.6 to 1.4 ng/ml.

Chronic 1,25$(OH)_2D_3$ administration produced a dose-dependent increase ($P < 0.001$) in the serum concentration of calcium but had little effect (with the exception of the 3-d time point) on the serum concentration of inorganic phosphate (P_i) (Table I). At 3, 7, and 12 d after beginning infusion of 1,25$(OH)_2D_3$ serum calcium was significantly ($P < 0.001$) increased in the 50- and 75-pmol/d dose groups. In the 25-pmol/d dose groups, although there was a trend for serum calcium to be increased, the difference did not reach significance ($P = 0.250$).

To determine if the reduction in serum 25-OH-D_3 brought

Table I. Effects of Chronic 1,25$(OH)_2D_3$ Infusion on the Serum Concentrations of 1,25$(OH)_2D_3$, 25-OH-D_3, and 24,25$(OH)_2D_3$, Ca, and P_i

Days after beginning infusion	1,25$(OH)_2D_3$ dose	Serum concentrations* of 1,25$(OH)_2D_3$	25-OH-D_3	24,25$(OH)_2D_3$	Ca	P_i
	pmol/d	*pg/ml*	*ng/ml*	*ng/ml*	*mg/dl*	*mg/dl*
3	0	113±15	21±3	—	10.0±0.1	7.8±0.3
	25	132±8‡	20±2	—	10.2±0.2	8.2±0.2§
	50	159±6‡	20±2	—	10.5±0.2‖	8.3±0.1§
	75	180±10‡	16±2	4.3±0.6	10.8±0.3‖	8.7±0.2§
7	0	103±9	20±2	—	10.4±0.2	7.6±0.3
	25	126±4‡	13±2¶	—	10.5±0.1	7.7±0.1
	50	166±10‡	11±1¶	—	11.0±0.2‖	8.2±0.2
	75	180±13‡	10±1¶	1.2±0.1	11.1±0.3‖	7.8±0.3
12	0	100±10	18±2	4.8±0.7	10.3±0.1	7.7±0.3
	25	124±6‡	12±1¶	4.6±0.4	10.5±0.3	7.5±0.1
	50	150±14‡	9±1¶	1.8±0.1**	10.7±0.2‖	7.7±0.1
	75	175±10‡	9±1¶	1.3±0.3**	11.2±0.1‖	7.5±0.2

* Mean±SE, six rats per group. ‡ Significantly higher than 0 dose group at all times, $P < 0.05$, multivariate analysis, Tukey test. § Significantly higher than equivalent dose groups from other days, $P < 0.05$, multivariate analysis. ‖ Significantly higher than 0 dose group, $P < 0.001$, multivariate analysis, Tukey test. ¶ Significantly lower than 0 dose group, $P < 0.001$, multivariate analysis, Tukey test. ** Significantly lower than in 0 dose group, $P < 0.001$, analysis of variance, Dunnett's test.

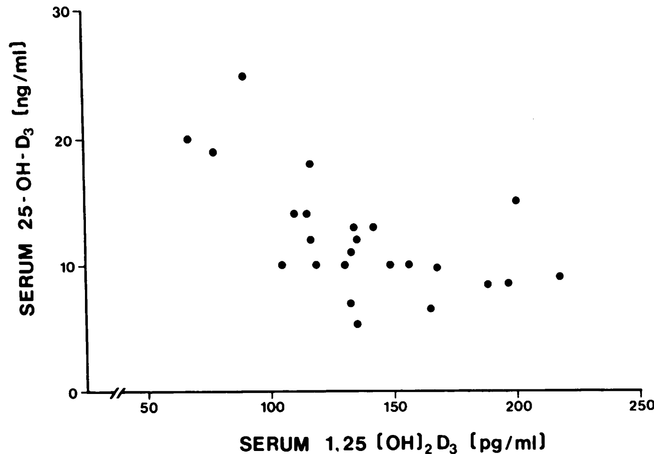

Figure 1. Relationship between the serum concentrations of 25-OH-D_3 and $1,25(OH)_2D_3$ in rats continuously infused (Alza osmotic minipumps) with $1,25(OH)_2D_3$ at the rate of 0, 25, 50, or 75 pmol/d for 12 d and fed a diet containing 4.41 U vitamin D_3/d, 1.2% Ca and 0.99% P.

about by chronic $1,25(OH)_2D_3$ administration was a result of an increase in metabolic clearance of 25-OH-D_3 or a decrease in production, we directly measured the MCR of 25-OH-D_3 using the constant infusion method. Chronic $1,25(OH)_2D_3$ administration increased the metabolic clearance of 25-OH-D_3 (Table II). In animals fed a synthetic diet containing 1.2% Ca, 1.2% P, and 100 U D_3/d, administration of $1,25(OH)_2D_3$ at the rate of 75 pmol/d for 12 d increased serum $1,25(OH)_2D_3$ from 140±6 to 199±6 pg/ml ($P < 0.005$), decreased serum 25-OH-D_3 from 24±1 to 14±2 ng/ml ($P < 0.001$), increased the MCR of 25-OH-D_3 from 17.5±0.6 to 29.7 μl/min ($P < 0.001$) but did not affect the PR of 25-OH-D_3.

To examine the mechanisms for the increased clearance of

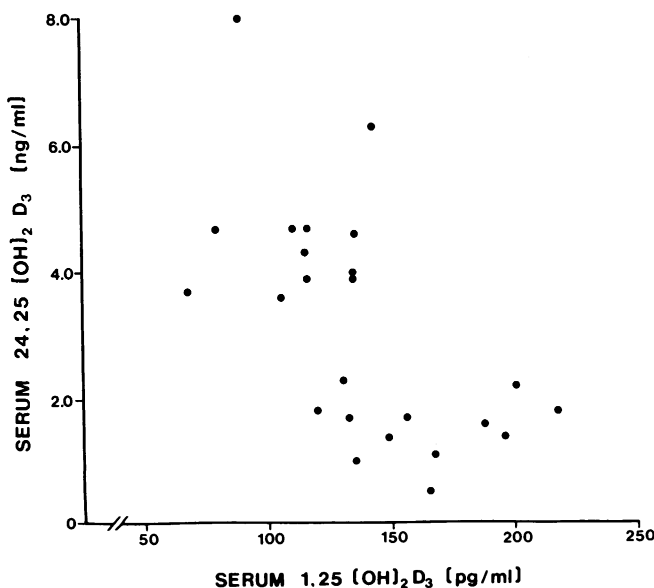

Figure 2. Relationship between the serum concentrations of $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ in rats continuously infused (Alza osmotic minipumps) with $1,25(OH)_2D_3$ at the rate of 0, 25, 50, or 75 pmol/d for 12 d and fed a diet containing 4.41 U vitamin D_3/d, 1.2% Ca and 0.99% P.

Table II. Effects of Chronic $1,25(OH)_2D_3$ Infusion for 12 d on the Serum Concentration, MCR, and PR of 25-OH-D_3

$1,25(OH)_2D_3$ dose	Serum concentration* of 25-OH-D_3	MCR*	PR*
pmol/d	*ng/ml*	*ul/min*	*pg/min*
0	24±1	17.5±0.6	421±29
75	14±2‡	29.7±1.8‡	410±30

* Mean±SE, six rats per group.
‡ $P < 0.001$, Student's *t* test, two-tailed.

25-OH-D_3 following $1,25(OH)_2D_3$ infusion, we evaluated the effect of chronic $1,25(OH)_2D_3$ administration in vivo on the metabolism of 25-OH-D_3, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ in liver and kidney homogenates. Chronic $1,25(OH)_2D_3$ administration (12 d, 75 pmol/d) resulted in virtually no change in the in vitro metabolism of 25-OH-D_3, $24,25(OH)_2D_3$, and $1,25(OH)_2D_3$ by liver homogenates (Table III). On the contrary, $1,25(OH)_2D_3$ administration (12 d, 75 pmol/d) substantially increased the in vitro metabolic conversion of 25-OH-D_3 to $24,25(OH)_2D_3$ in renal homogenates. In preparations of renal tissue from animals infused with vehicle alone, 89.4% of the original 25-OH-D_3 added to the incubation media was recovered intact, while 3.1% was converted to $24,25(OH)_2D_3$. In preparations of renal tissue from animals chronically infused with $1,25(OH)_2D_3$ for 12 d at a rate of 75 pmol/d, only 71.7% of the original 25-OH-D_3 added to the incubation media was recovered intact while 21.6% was converted to $24,25(OH)_2D_3$. Catabolism of $24,25(OH)_2D_3$ in renal preparations in two of three independent experiments was similar in vehicle- and $1,25(OH)_2D_3$-infused animals. In the third experiment, an increase in catabolism was observed. The reason for this anomaly is not clear. Catabolism of $1,25(OH)_2D_3$, which was very rapid in control animals, was further increased in animals chronically infused with $1,25(OH)_2D_3$. Kidney concentrations of endogenous 25-OH-D in vehicle and $1,25(OH)_2D_3$ infused animals were 0.7 ng/ml homogenate and 0.2 ng/ml homogenate (mean of duplicate determinations on pooled tissue homogenates), respectively. These concentrations of endogenous 25-OH-D are at least two orders of magnitude less than the Michaelis constant (K_m) of the 1- and 24-hydroxylase for 25-OH-D (21). Liver concentrations of endogenous 25-OH-D in vehicle- and infused animals were 0.4 ng/ml homogenate and 0.5 ng/ml homogenate, respectively. Liver and kidney concentrations of $24,25(OH)_2D$ and $1,25(OH)_2D$ in vehicle- and $1,25(OH)_2D_3$-infused animals were all ≤100 pg/ml of homogenate. These concentrations are <15% of the concentration of added substrate.

Because infusion of $1,25(OH)_2D_3$ increases the serum concentrations of both $1,25(OH)_2D_3$ and calcium, it is difficult to determine whether the increased serum concentration of $1,25(OH)_2D_3$ or the accompanying hypercalcemia or both are responsible for the decreases in serum 25-OH-D_3 and $24,25(OH)_2D_3$. To differentiate among these possibilities, animals were fed a synthetic vitamin D–deficient diet supplemented with 100 U of vitamin D_3/d and containing 1.2% phosphorus and either 0.4, 1.2, or 2.4% calcium in order to modulate the changes in serum calcium brought about by $1,25(OH)_2D_3$ infusion. Animals from each diet group were infused with either vehicle alone or $1,25(OH)_2D_3$ at the rate of 75 pmol/d for 12 d.

Table III. In Vitro Metabolism of 25-OH-D_3, 24,25$(OH)_2D_3$, and 1,25$(OH)_2D_3$ by Liver and Kidney Homogenates from Rats Chronically Infused with 1,25$(OH)_2D_3$ for 12 d

Tissue	Substrate	1,25$(OH)_2D_3$ dose	Percent substrate remaining or conversion to other metabolites* 25-OH-D_3	24,25$(OH)_2D_3$	1,25$(OH)_2D_3$	Other‡	Aqueous§
		pmol/d					
Liver	25-OH-D_3	0	89.1	0	0	5.0	2.0
		75	91.2	0	0	3.7	1.5
	24,25$(OH)_2D_3$	0	0	67.3	0	22.7	4.6
		75	0	69.3	0	26.5	2.2
	1,25$(OH)_2D_3$	0	0	0	96.9	1.6	2.4
		75	0	0	97.8	.8	2.0
Kidney	25-OH-D_3	0	89.4	3.1	0.2	0	2.9
		75	71.7	21.6	0.2	0	4.1
	24,25$(OH)_2D_3$	0	0	75.7	0	13.0	9.0
		75	0	80.2	0	14.2	11.3
	1,25$(OH)_2D_3$	0	0	0	6.0	5.5	62.5
		75	0	0	0	0	86.6

* Values (mean of duplicate determinations) are expressed as a percentage of the total counts incubated with the homogenates. ‡ Other unidentified metabolites. § Percent counts remaining in the aqueous phase after extraction.

Animals infused with 1,25$(OH)_2D_3$ and receiving the 2.4% calcium diet were slightly smaller than animals in the other groups (Table IV). The serum concentration of calcium in vehicle-infused animals was identical (10.9±0.1 mg/dl) when dietary calcium was 1.2 and 2.4%, but was lower (10±0.1 mg/dl, $P < 0.001$) when dietary calcium was reduced to 0.4%. 1,25-Dihydroxyvitamin D_3 infusion increased ($P < 0.001$) serum calcium by 1.0 to 1.1 mg/dl when dietary calcium was 1.2 and 2.4% but did not significantly change serum calcium when dietary calcium was 0.4%. The serum concentration of phosphate decreased significantly when dietary calcium was increased ($P < 0.25$) and/or when 1,25$(OH)_2D_3$ was infused ($P < 0.005$).

The serum concentration of 1,25$(OH)_2D_3$ in vehicle-infused animals decreased from 152±7 pg/ml to 121±pg/ml ($P < 0.001$) when dietary calcium was increased from 0.4% to 2.4% (Table IV). 1,25-Dihydroxyvitamin D_3 infusion increased ($P < 0.005$) serum 1,25$(OH)_2D_3$ within each of the diet groups, although the increment in increase was substantially less in the 0.4% dietary calcium group (23 pg/ml) than in the 2.4% dietary calcium group (61 pg/ml).

The serum concentration of 25-OH-D_3 in vehicle infused animals was identical (24±ng/ml) when dietary calcium was 0.4 and 1.2% but was higher (36±ng/ml, $P < 0.001$) when dietary calcium was increased to 2.4% (Table IV). Regardless of the concentration of 25-OH-D_3 in vehicle infused animals however, chronic 1,25$(OH)_2D_3$ infusion reduced serum 25-OH-D_3 ($P < 0.001$) approximately the same amount (viz. 10–14 ng/ml) in each diet group.

The serum concentration of 24,25$(OH)_2D_3$ in vehicle infused animals was similar, 10.9±0.6 and 11.4±1.1 ng/ml, when dietary calcium was 1.2 and 2.4%, respectively, but was lower, 6.8±0.9 ng/ml ($P < 0.001$), when dietary calcium was reduced to 0.4% (Table IV). In each dietary group, chronic 1,25$(OH)_2D_3$ infusion significantly reduced serum 24,25$(OH)_2D_3$ ($P < 0.001$).

Table IV. Effects of Dietary Calcium and Chronic 1,25$(OH)_2D_3$ Infusion for 12 d on Body Weights and the Serum Concentrations of 25-OH-D_3, 24,25$(OH)_2D_3$, 1,25$(OH)_2D_3$, Ca, and P_i

Dietary Ca	1,25$(OH)_2D_3$ dose	Body weight*	Serum concentrations* of 1,25$(OH)_2D_3$	25-OH-D_3	24,25$(OH)_2D_3$	Ca	P_i
%	*pmol/d*	*g*	*pg/ml*	*ng/ml*	*ng/ml*	*mg/dl*	*mg/dl*
0.4	0	238±4	151±7	24±1	6.8±0.9	10.5±0.1	7.6±0.1
	75	234±7	170±8‡	12±1§	2.0±0.2§	10.8±0.2	7.0±0.5
1.2	0	228±9	140±6	24±1	10.9±0.6	10.9±0.1	7.2±0.3
	75	235±7	199±6‡	14±2§	3.8±0.3§	12.0±0.1‡	6.5±0.3
2.4	0	226±7	121±5	36±7	11.4±1.1	10.9±0.1	7.0±0.5
	75	208±8‖	182±9‡	22±1§	3.0±0.3§	11.9±0.3‡	5.8±0.3§

* Mean±SE, six rats per group. ‡ Significantly higher than 0 dose group, $P < 0.005$, multivariate analysis, Tukey test. § Significantly lower than 0 dose group, $P < 0.005$, multivariate analysis, Tukey test. ‖ Significantly lower than 0 dose group, $P < 0.025$, multivariate analysis, Tukey test.

Discussion

Chronic $1,25(OH)_2D_3$ administration increased the serum concentration of $1,25(OH)_2D_3$ and reduced, in a dose- and time-dependent fashion, the serum concentrations of 25-OH-D_3 and $24,25(OH)_2D_3$. These results confirm previous reports (8, 11) that $1,25(OH)_2D_3$ can decrease serum 25-OH-D and extend these observations by defining the time course, calcium dependence, and dose-response effects of $1,25(OH)_2D_3$ on the serum concentrations of both 25-OH-D_3 and $24,25(OH)_2D_3$. Furthermore, our results indicate that in the rat, the decrease in serum 25-OH-D brought about by chronic $1,25(OH)_2D_3$ administration is due to an increase in the metabolic clearance of 25-OH-D_3 and not to a decrease in production of 25-OH-D_3. Whether shorter term infusions of $1,25(OH)_2D$ will alter production of 25-OH-D remains to be determined.

The increase in metabolic clearance of 25-OH-D_3 may in part be explained by the influence of $1,25(OH)_2D_3$ on the metabolism of 25-OH-D_3 in the kidney. Chronic $1,25(OH)_2D_3$ administration increased the in vitro conversion of 25-OH-D_3 to $24,25(OH)_2D_3$ in renal homogenates by approximately sevenfold, an effect consistent with previous reports demonstrating the stimulatory effect of $1,25(OH)_2D_3$ on the renal 25-hydroxyvitamin-D-24-hydroxylase (24-hydroxylase) (22–27). The increase in turnover of 25-OH-D to $24,25(OH)_2D$ can not be attributed to differences in tissue levels of endogenous 25-OH-D. Kidney homogenate concentrations of endogenous 25-OH-D in vehicle and $1,25(OH)_2D$ infused rats were 0.7 and 0.2 ng/ml, respectively, or 1.7 and 0.5×10^{-9} M, respectively, whereas the K_m for the 24-hydroxylase is 3.0×10^{-7} M (21). The increase in 24-hydroxylase activity would be expected to produce an increase in the turnover of 25-OH-D_3 to $24,25(OH)_2D_3$ in vivo and in turn result in a fall in circulating 25-OH-D_3. If this were the only metabolic change induced by $1,25(OH)_2D_3$ administration, however, one would expect to see an increase in the serum concentration of $24,25(OH)_2D_3$. This did not occur. In fact, the serum concentration of $24,25(OH)_2D_3$ decreased, and to a greater percentage than the serum concentration of 25-OH-D_3 (73% as compared with 50%, Table I, day 12). This suggests that if the fall in serum 25-OH-D_3 is due only to an increase in turnover to $24,25(OH)_2D_3$, then the MCR of $24,25(OH)_2D_3$ must also be increased, a point currently under investigation in our laboratory.

It is also conceivable that $1,25(OH)_2D_3$ administration increases the metabolic turnover of 25-OH-D_3 through pathways other than 24-hydroxylation. For example, it is possible that $1,25(OH)_2D_3$ may increase catabolism of 25-OH-D_3 in the liver. This concept is supported by the report from Baran et al. indicating that $1,25(OH)_2D_3$ in the perfusate can reduce the accumulation of [^{3}H]25-OH-D_3 in livers perfused with ^{3}H-vitamin D_3 (10). Baran et al. interpret their data to suggest that $1,25(OH)_2D_3$ decreases production of 25-OH-D_3. However, their results are also consistent with a $1,25(OH)_2D_3$-induced increase in catabolism of 25-OH-D_3. Whereas our data (Table IV) provide no evidence for increased catabolism of 25-OH-D_3 in liver homogenates from $1,25(OH)_2D_3$ infused rats, it is possible that intact liver tissue with a functioning biliary system is required for the catabolic pathway to be operative.

Although it is clear that $1,25(OH)_2D_3$ administration increases the MCR of 25-OH-D_3, it is not clear whether or not $1,25(OH)_2D_3$ acts directly to increase metabolism of 25-OH-D_3 or indirectly through changes in other systemic factors (e.g., calcium or parathyroid hormone, PTH). Since $1,25(OH)_2D_3$ administration causes hypercalcemia, it is possible that the changes in the serum concentrations of 25-OH-D_3 and $24,25(OH)_2D_3$ are brought about by the hypercalcemia or the presumed decrease in circulating PTH and not the increase in serum $1,25(OH)_2D_3$ per se. To examine the influence of serum calcium on the changes in serum 25-OH-D_3 and $24,25(OH)_2D_3$ induced by $1,25(OH)_2D_3$, we varied dietary calcium to modulate the changes in serum calcium brought about by $1,25(OH)_2D_3$ infusion. 1,25-Dihydroxyvitamin D_3 infusion increased serum calcium when dietary calcium was 1.2 and 2.4% but did not increase serum calcium when dietary calcium was 0.4%. Regardless of whether or not hypercalcemia was induced, however, $1,25(OH)_2D_3$ infusion reduced the serum concentrations of 25-OH-D_3 and $24,25(OH)_2D_3$. This suggests that an increase in serum calcium is not required for $1,25(OH)_2D_3$ infusion to reduce circulating 25-OH-D_3 and $24,25(OH)_2D_3$. These experiments, however, do not rule out the possibility that the presumptive fall in serum PTH, which likely occurs during $1,25(OH)_2D_3$ infusion, is in some way influencing metabolic clearance of 25-OH-D_3 and $24,25(OH)_2D_3$.

In summary, we have shown that chronic $1,25(OH)_2D_3$ administration decreases the serum concentration of 25-OH-D_3 by increasing the metabolic clearance of 25-OH-D_3 and not be decreasing its production. We have also demonstrated that this phenomenon is not dependent on the induction of hypercalcemia. Furthermore, we have shown that chronic $1,25(OH)_2D_3$ administration in vivo increases the in vitro conversion of 25-OH-D_3 to $24,25(OH)_2D_3$ but at the same time decreases circulating $24,25(OH)_2D_3$.

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