ABSENCE OF REGULATORY EFFECTS OF 1α,25-DIHYDROXYVITAMIN D_3 ON 25-HYDROXYVITAMIN D_3 METABOLISM IN RATS CONSTANTLY INFUSED WITH PARATHYROID HORMONE

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Summary: The regulatory role of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] in metabolism of 25-hydroxyvitamin D_3 was studied in sham-operated (sham) or thyroparathyroidectomized (TPTX) vitamin D-deficient rats into which calcium and parathyroid hormone (PTH) were constantly infused. A single dose of 325 or 650 pmol of 1α,25-$(OH)_2$-D_3 caused significant inhibition of 1α,25-$(OH)_2$-D_3 synthesis in D-deficient sham rats. This inhibition by 1α,25-$(OH)_2$-D_3, however, was not observed in D-deficient TPTX rats into which PTH was constantly infused. These results can be explained by supposing that the major regulatory effect of 1α,25-$(OH)_2$-D_3 on 1α,25-$(OH)_2$-D_3 synthesis is realized mostly, if not all, by suppressing endogenous secretion of PTH.

Much attention has been focused on the coordinate metabolism of 25-hydroxyvitamin D_3 (25-OH-D_3) to an active form, 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] or, alternatively, to an allegedly inert metabolite, 24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2$-D_3] in the kidney (1). These steps in 25-OH-D_3 metabolism are under strict control by the serum calcium concentration (2) through parathyroid hormone (PTH) (3-5). However, this type of regulation is minimal or absent in severe vitamin D deficiency, suggesting that some form of vitamin D is involved in this regulation (2,6). Vitamin D depresses renal biosynthesis of 1α,25-$(OH)_2$-D_3 and enhances that of 24,25-$(OH)_2$-D_3 (6). Tanaka and DeLuca (7,8) demonstrated that 1α,25-$(OH)_2$-D_3 itself is probably responsible for this type of regulation. Since PTH and 1α,25-$(OH)_2$-D_3 are both potent calcium-mobilizing hormones and the serum calcium concentration is precisely controlled, there may be an efficient feedback regulation of PTH secretion by the serum level of 1α,25-$(OH)_2$-D_3.

Abbreviations used: 25-OH-D_3, 25-hydroxyvitamin D_3; 1α,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; 24,25-$(OH)_2$-D_3, 24,25-dihydroxyvitamin D_3; PTH, parathyroid hormone; TPTX, thyroparathyroidectomy or thyroparathyroidectomized; sham, sham-operated.

Recently, Henry and Norman (9) reported that after injection of [^{3}H]-1α,25-$(OH)_2$-D_3 or [^{3}H]-25-OH-D_3, [^{3}H]-1α,25-$(OH)_2$-D_3 accumulates in chick parathyroid glands at a level equivalent to that in the target intestine. Brumbaugh et al. (10) isolated specific binding components for 1α,25-$(OH)_2$-D_3 from the cytoplasm and nucleus of chick parathyroid glands. Moreover, Oldham and coworkers (11) isolated a calcium-binding protein from normal porcine parathyroid tissue. Finally, Chertow et al. (12) demonstrated from both *in vivo* and *in vitro* studies that physiological levels of 1α,25-$(OH)_2$-D_3 inhibit PTH secretion. These findings indicate the existence of a negative feedback mechanism, in which PTH stimulates the synthesis of 1α,25-$(OH)_2$-D_3 which in turn inhibits PTH secretion (12).

In this study, we attempted to clarify quantitatively the relationship between PTH and 1α,25-$(OH)_2$-D_3 in the regulation of 25-OH-D_3 metabolism, and obtained the results suggesting that 1α,25-$(OH)_2$-D_3 exerts its regulatory effects mostly by inhibiting endogenous PTH secretion.

MATERIALS AND METHODS

Male weanling rats (Sprague-Dawley) were given a synthetic vitamin D deficient diet (13) containing 0.45% calcium and 0.3% phosphorus for 6 weeks. Then they were either sham-operated (sham) or thyroparathyroidectomized (TPTX) under anesthesia with hexobarbital (100 mg per kg body weight, i.p.). The left femoral artery and femoral vein were cannulated with heparinized polyethylene tubing (Intramedic 7400), and 300 μl of blood were collected from the femoral artery. Each animal was loosely fixed in a Ballman cage in a plastic chamber controlled at 24°C and a humidity of about 50%. The animals were then infused at a speed of 3 ml per hour via the cannulated femoral vein with a nutrient solution composed of 5 mM $CaCl_2$, 5 mM $MgCl_2$, 20 mM NaCl, 2.5 mM KCl and 4% glucose (14,15) unless otherwise stated. In some experiments, graded amounts (1-7.5 U/hr) of bovine PTH (TCA powder, Wilson Laboratories, Chicago, USA, 218 USP units/mg) were infused into TPTX rats immediately after the surgical treatment. Six hours later, vehicle (0.05 ml of ethanol) or 325-650 pmol of 1α,25-$(OH)_2$-D_3 (a gift from Dr. Matsunaga, Chugai Pharmaceutical Co.) was given via the cannulated femoral vein. Then 18 hours later, 300 μl of blood were again collected from the femoral artery and 0.5 μCi (50 pmol) of [26,27-^{3}H]-25-OH-D_3 (Radiochemical Centre, Amersham) was immediately injected via the femoral vein. The animals were killed 6 hours later and their blood was collected. The plasma was extracted by the method of Bligh and Dyer (16). The chloroform layers of the extracts were chromatographed on a Sephadex LH-20 column (1.5 x 30 cm) using 65% chloroform - 35% hexane as solvent (17). Radioactivity was measured in a Packard Tri-Carb, Model 3385 liquid scintillation spectrometer. Radioactive 1α,25-$(OH)_2$-D_3 and 24,25-$(OH)_2$-D_3 were identified by periodate cleavage (18) and liquid-liquid partition cochromatography with authentic materials on a Celite column (19). Serum Ca and P were measured as described elsewhere (6).

RESULTS AND DISCUSSION

The peak of material suspected to be [^{3}H]-1α,25-(OH)$_2$-D$_3$ from the Sephadex LH-20 column comigrated with synthetic 1α,25-(OH)$_2$-D$_3$ on a Celite column. In addition, it was not sensitive to periodate cleavage. The material suspected to be [^{3}H]-24,25-(OH)$_2$-D$_3$ eluted from the Sephadex LH-20 column was resolved into one major fraction (93% of the total radioactivity) and one minor fraction (7% of the radioactivity) on a Celite column. The material in the major peak lost more than 90% of its tritium on treatment with periodate overnight. Therefore, all the material in the peak suspected to be [^{3}H]-1α,25-(OH)$_2$-D$_3$ from the Sephadex LH-20 column was actually 1α,25-(OH)$_2$-D$_3$, and at least 80% of the material suspected to be [^{3}H]-24,25-(OH)$_2$-D$_3$ from the Sephadex LH-20 column was considered to be 24,25-(OH)$_2$-D$_3$.

Continuous calcium infusion first enabled vitamin D-deficient TPTX animals to survive for a considerable period of time. This system is extremely useful for quantitative evaluation of the role of PTH or 1α,25-(OH)$_2$-D$_3$ in the regulation of 25-OH-D$_3$ metabolism. TPTX suppressed [^{3}H]-1α,25-(OH)$_2$-D$_3$ synthesis to one-fourth of the control level 24 hours after the surgical treatment, but did not enhance [^{3}H]-24,25-(OH)$_2$-D$_3$ production (Table I). The suppressed level of 1α,25-(OH)$_2$-D$_3$ synthesis was maintained for at least 48 hours after surgery (data are not shown). Graded doses of PTH constantly infused into D-deficient TPTX rats induced dose-dependent stimulation of [^{3}H]-1α,25-(OH)$_2$-D$_3$ synthesis (Fig 1). This confirms previous reports that PTH stimulates the 1α-hydroxylation reaction of 25-OH-D$_3$ (3-5).

Injection of a single dose of 650 pmol of 1α,25-(OH)$_2$-D$_3$ into D-deficient TPTX rats 18 hours before administration of [^{3}H]-25-OH-D$_3$ caused further inhibition of [^{3}H]-1α,25-(OH)$_2$-D$_3$ synthesis and concomitant marked stimulation of [^{3}H]-24,25-(OH)$_2$-D$_3$ synthesis (Table I). These results suggest that, though PTH certainly stimulates 1α,25-(OH)$_2$-D$_3$ synthesis, some part of the 1α-hydroxylation reaction is not directly dependent on PTH, but closely related to induction of the 24-hydroxylation reaction which takes place in the presence of

Table I Effects of 1α,25-$(OH)_2$-D_3 on 25-OH-D_3 metabolism and serum Ca and P levels in sham and TPTX rats

	1α,25-$(OH)_2$-D_3 predosed	[^{3}H]-1α,25-$(OH)_2$-D_3 produced	[^{3}H]-24,25-$(OH)_2$-D_3 produced	Serum Ca		Serum P	
				before infusion	after 24 hrs' infusion	before infusion	after 24 hrs' infusion
	pmol	pmol/dl serum		mg/dl		mg/dl	
Sham	0	18.4 ± 0.3 (100%)	0.3 ± 0.3	5.9 ± 0.5	8.0 ± 0.1	9.9 ± 0.8	8.6 ± 0.9
	325	15.3 ± 0.3 (83%)	1.8 ± 0.3	6.6 ± 0.9	9.8 ± 0.5	9.1 ± 0.9	8.6 ± 1.2
	650	12.5 ± 2.0 (68%)	2.0 ± 0.9	5.3 ± 0.3	9.3 ± 0.4	10.0 ± 0.4	8.2 ± 0.9
TPTX	0	4.4 ± 0.5 (24%)	0.9 ± 0.5	5.5 ± 0.4	4.5 ± 0.2	8.5 ± 1.2	12.0 ± 1.0
	650	1.5 ± 0.6 (8%)	12.1 ± 1.1	5.1 ± 1.0	5.8 ± 0.7	9.6 ± 0.1	13.9 ± 1.6

Values are means of those in 4 independent experiments ± standard errors.

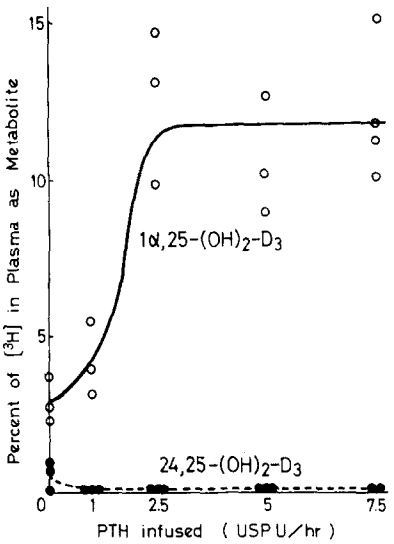

Fig 1. Effects of various doses of PTH on 25-OH-D_3 metabolism. PTH was infused into vitamin D-deficient TPTX rats at the rate indicated on the abscissa and the rate of 25-OH-D_3 metabolism was shown on the ordinate as percentage of the total radioactivity in the plasma.

1α,25-$(OH)_2$-D_3 and absence of PTH. Injection of the same dose of 1α,25-$(OH)_2$-D_3 into D-deficient sham rats, however, did not enhance the production of [^{3}H]-24,25-$(OH)_2$-D_3, but significantly inhibited [^{3}H]-1α,25-$(OH)_2$-D_3 synthesis (Table I).

To test whether inhibition of [^{3}H]-1α,25-$(OH)_2$-D_3 synthesis by 1α,25-$(OH)_2$-D_3 is due to suppression of PTH secretion, a single dose of 650 pmol of 1α,25-$(OH)_2$-D_3 was given to D-deficient TPTX rats into which graded amounts of PTH were constantly infused (Table II). Production of [^{3}H]-1α,25-$(OH)_2$-D_3 was not suppressed by prior dose of 1α,25-$(OH)_2$-D_3 in TPTX rats infused with PTH. When PTH was infused at a rate of 1 U/hr, a dose of 650 pmol of 1α,25-$(OH)_2$-D_3 caused a small but significant increase in [^{3}H]-24,25-$(OH)_2$-D_3 synthesis. However, this stimulation was not observed in TPTX rats infused with 7.5 U/hr of PTH. Constant infusion of 7.5 U/hr of PTH into D-deficient TPTX rats restored 1α,25-$(OH)_2$-D_3 synthesis to a level similar to that in D-deficient sham animals. This is consistent with our estimation that the basic secretion rate of PTH is

Table II Effects of 1α,25-$(OH)_2$-D_3 on 25-OH-D_3 metabolism in vitamin D-deficient TPTX rats infused with PTH

PTH infused	1α,25-$(OH)_2$-D_3	[^{3}H]-1α,25-$(OH)_2$-D_3	[^{3}H]-24,25-$(OH)_2$-D_3	Serum Ca		Serum P	
	predosed	produced	produced	before infusion	after 24 hrs' infusion	before infusion	after 24 hrs' infusion
USP U/hr	pmol	pmol/dl serum		mg/dl		mg/dl	
7.5	0	19.0 ± 1.7	0	5.4 ± 0.4	10.3 ± 1.2	9.3 ± 1.4	9.2 ± 0.8
	650*	17.4 ± 0.8	1.2 ± 0.9	5.6 ± 0.4	9.9 ± 0.7	9.2 ± 0.4	8.7 ± 0.7
1.0	0	6.5 ± 1.0	0.6 ± 0.2	4.7 ± 0.5	5.0 ± 1.1	8.2 ± 0.1	9.8 ± 0.2
	650	7.2 ± 0.1	3.2 ± 0.5	5.0 ± 0.6	7.1 ± 1.5	7.6 ± 0.8	11.7 ± 0.5

Values are means of those in 4 independent experiments ± standard errors.

* 2.5 mM $CaCl_2$ was infused into this group of rats to give serum levels of Ca and P comparable with those of the controls.

5.1-8.6 USP units/hr in D-deficient rats used in this experiment (submitted for publication).

Chertow et al. (12) suggested the existence of a feedback mechanism in which PTH stimulates the synthesis and secretion of 1α,25-$(OH)_2$-D_3, which in turn inhibits PTH secretion. Our results are consistent with their suggestion. The feedback regulation of the renal synthesis of 1α,25-$(OH)_2$-D_3 by 1α,25-$(OH)_2$ D_3, therefore, seems to occur mainly, if not entirely, by suppression of PTH secretion. Stimulation of the 24-hydroxylation reaction by 1α,25-$(OH)_2$-D_3 was only observed when PTH secretion was suppressed. Thus, it is most attractive to consider that 25-OH-D_3 metabolism in the kidney is under the control of finely balanced concentrations of serum PTH and 1α,25-$(OH)_2$-D_3. Intracellular events in renal cells induced by PTH and 1α,25-$(OH)_2$-D_3 are currently being investigated.

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