Inhibition of Parathyroid Hormone Secretion by 25-Hydroxycholecalciferol and 24,25-Dihydroxycholecalciferol in the Dog

JANET M. CANTERBURY, SAM LERMAN, ALICE J. CLAFLIN, HELEN HENRY, ANTHONY NORMAN, and ERIC REISS, *Department of Medicine, University of Miami School of Medicine, Miami, Florida 33152, and the Department of Biochemistry, University of California at Riverside, Riverside, California 92502*

ABSTRACT We studied the effects of vitamin D metabolites on parathyroid hormone (PTH) secretion. Test materials were injected into the cranial thyroid artery of the dog, and immunoreactive PTH was measured frequently in serum samples from the inferior thyroid vein and the femoral vein. This model for the study of secretion had previously been validated with the use of known modulators on PTH secretion. In control experiments, injection of 100% ethanol, the vehicle in which cholecalciferol (D_3) metabolites were suspended, resulted in no change in PTH secretion. Likewise, native vitamin D_3, in doses ranging from 250 to 1,250 ng had no effect on PTH secretion. 25-Hydroxycholecalciferol, 25-(OH)D_3, in doses of 125–240 ng, caused complete suppression of PTH secretion. When 24,25-dihydroxycholecalciferol, 24,25-$(OH)_2D_3$, was injected in doses of 50–250 ng, suppression of PTH secretion was again complete; in doses of 5 ng, injection of this metabolite resulted in significant but incomplete suppression of secretion. In doses of 50–250 ng, 1,25-$(OH)_2D_3$ strongly stimulated PTH secretion, but in a dose of 5 ng this metabolite had no effects. Injection of equal doses of 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ resulted in significant suppression of PTH secretion. Hypocalcemia-induced stimulation of PTH secretion was suppressed by 24,25-$(OH)_2D_3$ while hypercalcemia-induced suppression of PTH secretion was stimulated by 1,25-$(OH)_2D_3$. In all experiments showing suppression of PTH secretion, peripheral PTH decreased. Arguments are presented for considering the suppressive effects of D_3 metabolites as physiologic modulators. However, this stimulating effect of 1,25-$(OH)_2D_3$ occurred only in pharmacologic doses and hence probably has no physiologic relevance.

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INTRODUCTION

While much is known about the independent actions of the two principal regulators of mammalian calcium homeostasis, parathyroid hormone (PTH)[1], and the most active metabolite of vitamin (D_3), 1,25-dihydroxycholecalciferol 1,25-$(OH)_2D_3$, limited information exists about their interrelationships (1). It has been known for many years that vitamin D is required for PTH to exert its full biologic action; in rachitic animals, the calcemic response to injected PTH is greatly blunted (1, 2). Recently, the converse of the interrelationship has been proposed: PTH stimulates conversion of 25-hydroxycholecalciferol, 25-(OH)D_3, to 1,25-$(OH)_2D_3$ in the kidney (3–5).

Previous work has established the existence of a metabolic pathway for conversion of D_3, first by the liver to 25-(OH)D_3, and then by the kidney to 1,25-$(OH)_2D_3$, or 24,25-dihydroxycholecalciferol (24,25-$(OH)_2D_3$) (6–9). The biologically most active form of D_3, 1,25-$(OH)_2D_3$ initiates the biological response of increased calcium absorption in the target tissues, intestine, and bone. The biologic functions of 24,25-$(OH)_2D_3$ remain to be elucidated.

Three lines of evidence support the concept of an action of 1,25-$(OH)_2D_3$ on the parathyroid glands: (*a*) the parathyroids selectively localize 1,25-$(OH)_2D_3$ in vivo (10); (*b*) cytoplasmic and nuclear binding of 1,25-

[1] *Abbreviations used in this paper*: 24,25-dihydroxycholecalciferol, 24,25-$(OH)_2D_3$; 25-hydroxycholecalciferol, 25-(OH)D_3; iPTH, immunoreactive parathyroid hormone; PTH, parathyroid hormone.

$(OH)_2D_3$ in parathyroid glands has been reported (11, 12), and (*c*) simultaneous administration of 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$, but not their separate administration, mediate a reduction of parathyroid gland weight and DNA content in rachitic chicks independent of any effects on serum calcium (13). In addition, calcium binding protein, similar to the one which is D-dependent and found in intestinal mucosa, has been reported to be present in porcine parathyroid glands (14).

Recently, more direct experiments to examine the influence of vitamin D on PTH secretion have been reported. Shen et al. presented preliminary evidence that 1,25-$(OH)_2D_3$ stimulates PTH secretion (15). Chertow et al. reported that 1,25-$(OH)_2D_3$ suppresses PTH secretion, both in vivo and in vitro (16). Most recently, Okano et al. found no effect of 1,25-$(OH)_2D_3$ nor of 24,25-$(OH)_2D_3$ on PTH secretion in vitro. However, when both metabolites were given together the secretion of PTH into the tissue culture was decreased (17). Llach et al. found essentially no change in serum PTH in response to the administration of 1,25-$(OH)_2D_3$ in man (18). In all of these studies, PTH secretion was assessed by measurement of immunoreactive parathyroid hormone (iPTH) in the peripheral circulation. Care and colleagues (19) approached the problem by measuring iPTH directly in the thyroid venous effluent of goats. They reported preliminary experiments showing suppression of PTH secretion by 24,25-$(OH)_2D_3$ and, in some experiments, stimulation of PTH secretion by 1,25-$(OH)_2D_3$.

Thus, while there exist suggestive data concerning a PTH secretion 1,25-$(OH)_2D_3$ negative feedback loop, the available information is fragmentary and inconsistent. The only part of this system that is firmly established by work from several laboratories is stimulation of 1-hydroxylation of 25-$(OH)D_3$ in the kidney by PTH. Theoretically, 1,25-$(OH)_2D_3$ should suppress PTH secretion. Otherwise, these two calcemic hormones could perpetuate each other's secretion or activation, and hypercalcemia could become unavoidable. The effects of 24,25-$(OH)_2D_3$ on PTH secretion cannot be predicted in this scheme because the role of this metabolite in calcium homeostasis has not been clearly defined.

With this background, we embarked on a systematic investigation of the effects of vitamin D metabolites on PTH secretion in vivo. The results unexpectedly demonstrated suppression of PTH secretion by 24,25-$(OH)_2D_3$.

METHODS

The surgical procedures employed will be described in detail elsewhere (20). In brief, mongrel dogs of both sexes weighing 15–20 kg were anesthetized with sodium pentobarbital, and catheters for blood sampling were placed into the right inferior thyroid vein and into a femoral vein. Injections of test materials were given directly into or near the cranial thyroid artery. In early experiments, it proved technically difficult to inject directly into the thyroid artery. Therefore materials were injected into the carotid artery just below the cranial thyroid artery while briefly clamping the carotid above it. In later experiments it was possible to cannulate the thyroid artery directly. Results obtained by these two procedures were indistinguishable. An advantage of using the dog for the study of PTH secretion is that in this species both parathyroid glands of one side of the neck drain predominantly into a single thyroid vein.

Blood samples for serum iPTH and calcium determinations were taken simultaneously from the femoral and inferior thyroid veins for a minimum of three base line samples, taken at 5–10-min intervals before injection of test material. Thereafter, the samples were taken from both sides every 5 min for the first 60 min and then every 15 min for 2–4 h.

The vitamin D metabolites used were chemically synthetized and crystalline. Upon analysis they exhibited typical ultraviolet spectra at 264 nM for vitamin D compounds. Each metabolite yielded a single component on Sephadex LH-20 (Pharmacia Fine Chemicals, Pharmacia, Inc., Piscataway, N. J.) and high pressure liquid chromatography.

Antiserum CH-71 at a final dilution of 1:125,000 was used for all iPTH measurements. This antiserum cross-reacts with intact hormone as well as NH_2- and COOH-terminal fragments (21–23). For phase separations, the dextran-coated charcoal method as described by Arnaud et al. was used (24). All samples were measured in duplicate at two widely varying dilutions. The coefficient of variation of replicates was 9%; assays showing a larger variation were repeated. iPTH levels reported are in arbitrary units (μl eq/ml), relating the potency of the test serum to that of a standard hyperparathyroid serum which was assigned a potency of 1,000 μeq/ml. The standard serum was obtained from a dog with experimentally induced chronic renal failure (25). Serum calcium was measured on fresh samples by flame emission spectrophotometry; in some experiments, ionized calcium was measured by use of the Orion flow-through electrode, model SS-20 (Orion Research, Cambridge, Mass.).

RESULTS

Control experiments

Vitamin D_3 and its metabolites were suspended in a vehicle of ethanol. Injection of this vehicle into the thyroid artery caused no changes in any of the parameters measured (Fig. 1). Further control experiments using native vitamin D_3 in large doses showed that the unaltered vitamin had no effect, the results being identical with those obtained with ethanol alone. In three experiments, vitamin D was infused in the dose of 250 ng. In two additional experiments the dose was 1,250 ng.

The model was very stable. Fluctuations in PTH secretion probably represent small bursts of secretion previously suggested by Deftos et al. (26). In all experiments, the concentration of iPTH in the thyroid venous effluent was 2–4 times higher than in the peripheral venous circulation. Such a gradient has been demonstrated in man at sites of hyperfunctioning para-

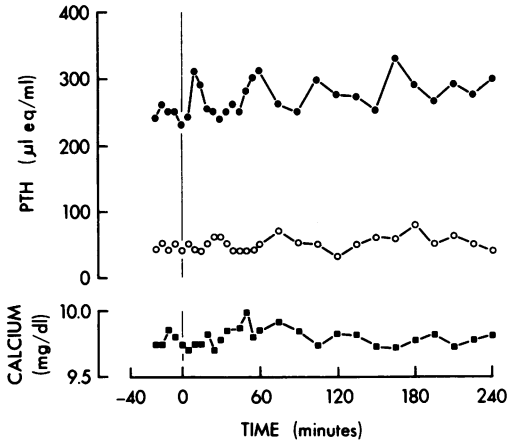

FIGURE 1 Effects of a bolus injection of 100% ethanol, the vehicle in which vitamin D metabolites were dissolved, into the thyroid artery. (● —— ●), iPTH in the thyroid venous effluent; (○ —— ○), iPTH in peripheral venous sample; ■ —— ■, serum calcium.

thyroid gland but not in veins draining normal parathyroid tissue (27).

Vitamin D metabolites

25-(OH)D_3 (Five experiments). A summary of results is given in Table I. Doses of 125 and 250 ng 25-(OH)D_3 caused prompt and essentially complete suppression of secretion. Suppression was noted at the earliest sampling time, 2–5 min after injection. This time course was indistinguishable from that observed with 24,25-$(OH)_2D_3$. Maximal effects were observed at 25–30 min. The time of maximal effect was remarkably constant with all metabolites studied, ranging on the average from 23 to 32 min. In these experiments as well as all others involving suppression of PTH secretion, return to base line was also quite reproducible, the average time for maximal suppression to resumption of base-line secretion ranging from 30 to 50 min. Decreased secretion of PTH was reflected in peripheral concentrations of iPTH. As expected, peripheral iPTH changes lagged 15–20 min behind changes in secretion, both with respect to suppression and to recovery.

In none of the experiments listed in the Table was there any significant change in the peripheral total or ionized calcium concentration.

24,25-$(OH)_2D_3$. 12 experiments were performed with this metabolite. In doses of 50–250 ng, suppression of secretion was complete. In the lowest dose used, 5 ng, suppression was unequivocal but incomplete.

Although the control experiments previously described effectively excluded artifacts produced by injections into the thyroid artery, administration of the metabolite into a peripheral vein provided an additional control. In three experiments with a dose of 2,500 ng, distinct but incomplete suppression of PTH was again observed.

Not shown in Table I are three experiments in which 24,25-$(OH)_2D_3$ was infused into the thyroid artery over a 20 min period, 50 ng/min. A representative experiment is shown in Fig. 2. PTH secretion was completely suppressed for 35–45 min after cessation of the infusion. Peripheral iPTH concentration decreased *pari passu* to unmeasurably low levels. During the recovery phase, PTH secretion increased above base line for a brief period. This phenomenon of a period of increased secretion above base line during the recovery phase was characteristic of all experiments in which complete suppression of secretion was attained. However, there was considerable variability in the degree of increase and its duration.

In contrast to experiments involving bolus injections, the infusion experiments showed a decrease in serum calcium when PTH secretion and peripheral iPTH concentrations became unmeasurably low.

1,25-$(OH)_2D_3$. 12 experiments were performed with this metabolite. In doses of 50–250 ng there resulted an unequivocal increase in PTH secretion with maximal effects occurring on the average within 24–30 min. In the lowest dose tested, 5 ng, this metabolite had no effect. The characteristic pattern of response is illustrated in Fig. 3. Stimulation of secretion was followed by a slow return toward control values and, subsequently, to a decrease substantially below control values. In contrast to the experiments showing suppression of PTH secretion, peripheral concentrations of iPTH surprisingly did not change in these experiments. A possible explanation is that in studies in which PTH secretion was stimulated, frequent sampling of the thyroid vein drained off the bulk of the increased PTH secreted.

Combination of metabolites. In three experiments, 250 ng each of 1,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ were injected simultaneously (Table I). In these doses, the metabolites used singly exerted maximal effects. In combination, these metabolites caused unequivocal but incomplete suppression that was reflected in declining peripheral iPTH concentrations.

Combined effects of calcium on vitamin D metabolites. The effects of vitamin D metabolites on PTH secretion were to some extent independent of the classic responses of PTH secretion mediated by perturbations in serum calcium. In Fig. 4A are shown representative results of 1,25-$(OH)_2D_3$ administration on PTH secretion in animals in whom PTH secretion was suppressed by hypercalcemia. 1,25-$(OH)_2D_3$ was injected 20 min after the initiation of an intravenous calcium infusion at a time when PTH secretion had decreased to very low levels. Despite a hypercalcemia of 11.5 mg/dl, 1,25-$(OH)_2D_3$ elicited a rapid

TABLE I

Summary of Results of Changes in PTH in Response to Infusion of Vitamin D_3 Metabolites

Vitamin D_3 metabolite	Experiment number	PTH thyroid venous effluent serum*				PTH peripheral serum*			
		Base line	Maximum change (Δ)	Time to maximum (Δ)	Time to base line	Base line PTH	Maximum change (Δ)	Time to maximum (Δ)	Time to base line
25-[OH]D_3 240 ng	1	360	−350	25	40	85	−80	45	65
	2	295	−295	20	45	75	−65	50	60
	3	220	−200	30	43	80	−75	45	65
	Mean±SE	292±40	−282±44	25±3	43±2	80±3	−73±4	47±2	63±2
25-[OH]D_3 125 ng	1	320	−300	25	40	85	−85	45	60
	2	210	−200	35	60	60	−50	65	80
	Mean	265	−250	30	50	73	−68	55	70
24,25-[OH]$_2D_3$ 250 ng	1	350	−350	20	35	80	−80	40	55
	2	300	−300	25	40	75	−60	30	60
	3	400	−375	20	35	80	−75	25	55
	4	360	−350	30	40	90	−90	40	60
	5	425	−400	30	50	90	−85	45	65
	Mean±SE	367±22	−355±17	25±2	40±3	83±3	−75±5	36±4	58±2
24,25-[OH]$_2D_3$ 50 ng	1	360	−350	20	30	80	−75	40	60
	2	300	−300	20	35	80	−80	30	50
	3	445	−395	25	40	95	−95	35	55
	4	285	−275	30	45	90	−85	40	55
	Mean±SE	348±36	−330±27	24±2	38±3	86±4	−84±4	36±3	55±2
24,25-[OH]$_2D_3$ 5 ng	1	295	−250	20	30	70	−55	40	50
	2	380	−200	25	35	75	−60	45	55
	3	240	−195	25	30	80	−60	45	60
	Mean±SE	272±17	−215±18	23±2	32±2	75±3	−58±2	43±2	55±3
24,25-[OH]$_2D_3$ into femoral vein 2,500 ng	1	310	−200	30	35	75	−45	45	60
	2	360	−180	30	35	90	−50	50	60
	3	220	−100	35	40	80	−40	50	65
	Mean±SE	297±41	−160±31	32±2	37±2	82±4	−45±3	48±2	62±2
1,25-[OH]$_2D_3$ 250 ng	1	320	+215	20	105	80	NC	—	—
	2	295	+225	25	75	85	NC	—	—
	3	250	+260	30	105	75	NC	—	—
	4	400	+220	20	60	90	NC	—	—
	5	420	+350	25	125	60	NC	—	—
	Mean±SE	337±32	+254±25	24±2	96±13	78±5			
1,25-[OH]$_2D_3$ 50 ng	1	360	+200	25	100	75	NC	—	—
	2	350	+185	30	90	85	NC	—	—
	3	395	+190	35	75	90	NC	—	—
	4	280	+175	30	85	70	NC	—	—
	Mean±SE	346±24	+187±6	30±2	88±5	80±5			
1,25-[OH]$_2D_3$ 5 ng	1	295	NC	—	—	60	NC	—	—
	2	400	NC	—	—	85	NC	—	—
	3	410	NC	—	—	90	NC	—	—
	Mean±SE	368±37				78±9			
1,25-[OH]$_2D_3$ & 24,25-[OH]$_2D_3$ 250 ng	1	350	−250	25	30	85	−70	40	55
	2	375	−300	25	30	90	−80	35	60
	3	320	−275	20	30	85	−75	30	55
	Mean±SE	348±16	−275±14	23±2	30	86±2	−75±3	37±4	57±2

In all experiments except the one identified, vitamin D metabolites were administered as a single bolus of 0.2 ml into the superior thyroid artery. In the injections into the femoral vein, the volume of injection was 1.5 ml. NC, no change.

* PTH levels are given as μl eq/ml. The range for normal dog peripheral samples is 70–95.

increase in PTH secretion. This phenomenon was observed in all three experiments of this type performed. There was some suggestion that the stimulatory effect of 1,25-$(OH)_2D_3$ was inversely related to the level of the serum calcium. In one experiment, in which the serum calcium rose to 15.5 mg/dl, 1,25-$(OH)_2D_3$ exerted only a minimal stimulatory effect on PTH secretion.

Representative results of three experiments in which 24,25-$(OH)_2D_3$ was injected into the thyroid artery of animals whose PTH secretion had previously been stimulated by hypocalcemia are shown in Fig. 4B. After 20 min of EDTA-induced hypocalcemia, PTH secretion was strongly stimulated. Despite persistent hypocalcemia, the injection of 24,25-$(OH)_2D_3$ promptly and completely suppressed PTH secretion.

DISCUSSION

The systematic investigation of PTH secretion in an experimental model permitting infusion of the substance to be studied into the thyroid artery and sampling of the thyroid venous effluent has important advantages. Care et al. has pioneered in the development of such models (28). If the study materials are given orally or intravenously, secondary metabolic

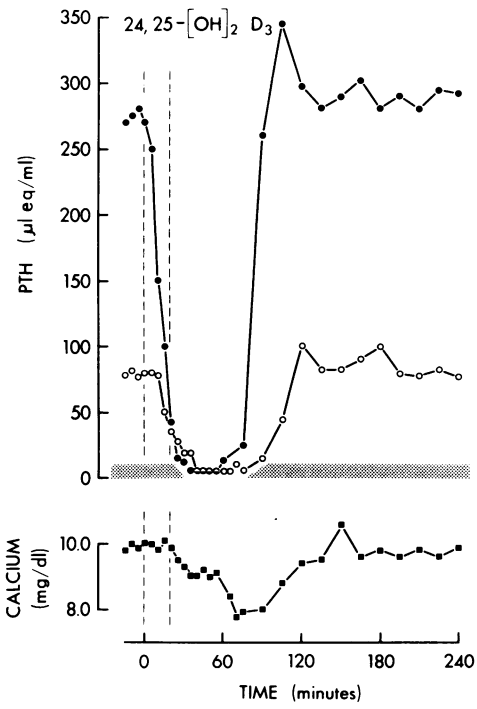

FIGURE 2 Effects of a continuing infusion of (24,25-$(OH)_2D_3$) 50 ng/min for 20 min on PTH in the thyroid venous effluent (● —— ●); peripheral iPTH (○ —— ○); and on peripheral serum calcium (■ —— ■).

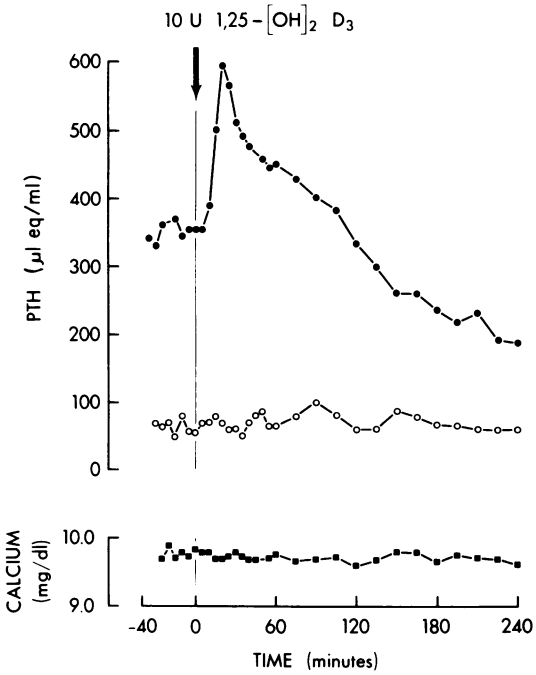

FIGURE 3 Effects of a bolus injection of 250 ng of (1,25-$(OH)_2D_3$) into the thyroid artery on iPTH in the thyroid venous effluent (● —— ●); peripheral iPTH (○ —— ○), and peripheral serum calcium (■ —— ■).

events can occur that may influence the actual or apparent hormone secretion. Our canine model has been validated by demonstrating an appropriate response to established modulators of PTH secretion. Hypocalcemia and infusions of catecholamines consistently stimulate PTH secretion while hypercalcemia suppresses it (20).

In the foregoing presentation of results, we have equated the concentration of iPTH in the thyroid venous effluent with PTH secretion. This is justified (*a*) if the blood flow remains substantially unaltered during the experiments, and (*b*) if the molecular species of secreted hormone is not altered. Although it is known that PTH can affect blood flow to several organs (29), the present results cannot be explained on the basis of changing flow rates. In several experiments the thyroid veins were severed and total venous effluent was measured before and after infusion of vitamin D metabolites. The amount of effluent remained constant. In addition, changes in blood flow would not explain the decreasing concentrations of hormone in some experiments with increasing concentrations in others.

At present, there is disagreement on the distribution of molecular species of PTH secreted by normal parathyroid glands and in hyperparathyroidism (30–32). However, there is agreement that the predominant

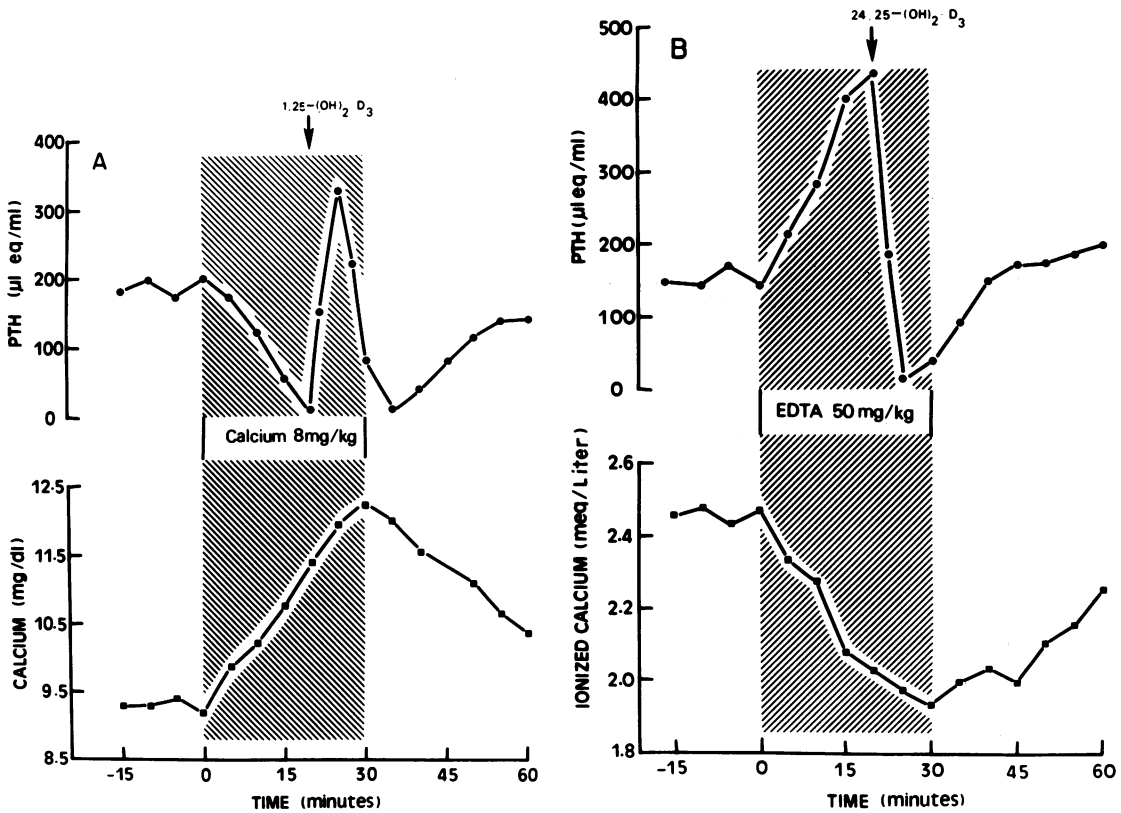

FIGURE 4 Effects of vitamin D metabolites on iPTH in thyroid venous effluent in the course of suppression and stimulation of PTH secretion by hypercalcemia and hypocalcemia, respectively. (A) 250 ng ($1,25\text{-}(OH)_2D_3$); (B) 250 ng ($24,25\text{-}(OH)_2D_3$).

molecular species secreted under all circumstances tested is the intact 1–84 amino acid peptide. In the present study, unconcentrated thyroid venous effluents were fractionated and immunoassayed by methods previously described (21). In one study, the sample fractionated was derived from an EDTA infusion at a time of maximal stimulation of PTH secretion; in another, a sample from an infusion of $1,25\text{-}(OH)_2D_3$ was fractionated. The results of these fractionations were equivalent with roughly 85% of immunoreactivity eluting in the position of the 1–84 amino acid peptide. Since the antiserum used in these studies possesses strong affinity for fragments, changing secretion of the molecular species of secreted hormone in the direction of secretion of fragments could not account for decreasing immunoreactivity observed in many of these experiments. Thus, the assumption that PTH secretion in the thyroid venous effluent can be equated with secretion seems adequately justified. On the other hand, we cannot exclude that the vitamin D metabolites had some influence on PTH metabolism after secretion. This possibility is suggested by the rapid decline of peripheral hormone concentration in some experiments. Our own previous experience and that reported in the literature would suggest the COOH-terminal fragments have a half-life considerably longer than that observed in the present experiments (30, 33).

It is impossible to know whether the doses of vitamin D metabolites used in these experiments were in the physiologic range or above it. The metabolites were diluted to an unknown extent during the course of injection.[2] One can estimate maximal concentrations by assuming no dilution. In this limiting case, the concentration of metabolites reaching the parathyroid glands would be five times the quantity listed in Table I. With respect to $25\text{-}(OH)D_3$, the lowest effective concentration tested would therefore be 625 ng/ml, which is roughly 25 times the reported physiologic concentration of this metabolite (34–36). However, since peripheral hormone concentration was virtually completely suppressed, the contralateral parathyroid glands must also have been suppressed. If

[2] A reasonable minimal estimate of the extent of dilution of injected materials is 1:10 based on the volume of distribution of the thyroid arterial bed. In addition, blood flow to the parathyroid glands represents only a very small fraction of flow to the thyroid.

one assumes dilution of the metabolite in the extracellular fluid or even in plasma, the effective concentration exerting an influence on the contralateral glands would be very low indeed. Limited information exists concerning the physiologic concentrations of 24,25-$(OH)_2D_3$. The reported mean concentration is 4 ng/ml with considerable variation from one assay to another (19, 37, 38).[3] Using the same assumptions previously mentioned, it is evident that the concentrations of this metabolite exerting a suppressive effect on the parathyroid glands may well be within the physiologic range. On the other hand, the effective concentration of 1,25-$(OH)_2D_3$ was far in excess of reported physiologic concentrations (36, 39, 40) regardless of what assumptions are made about dilution. The normal concentration of 1,25-$(OH)_2D_3$ is 500–1,000 times less than that of 24,25-$(OH)_2D_3$, and the minimal effective dose was large.

The observation of increased secretion for a short time after suppression would suggest that the primary effect of the metabolites was on secretion of hormone rather than synthesis; after the suppression of secretion was dissipated, performed hormone apparently was secreted in increased amounts. Similarly, in the case of 1,25-$(OH)_2D_3$, decrease of secretion below baseline values at later time periods may reflect depletion of preformed hormone in the glands. However, this explanation may not be tenable because decreased secretion was observed as long as 4 h after injection, and one would expect new hormone synthesis to occur by this time. Observation of late suppression of secretion is in accord with the previously mentioned experiments of Chertow et al. (16).

The mechanisms by which vitamin D metabolites influence PTH secretion are not known. Vitamin D, like all steroid hormones, is proposed to initiate biologic responses in target tissues as a consequence of the steroid's association with the cytoplasmic receptor, and then by transfer of the steroid-receptor complex to the nucleus of the cell (41). The steroid-receptor complex then initiates a synthesis of specific messenger RNA molecules (42, 43) which, in turn, code for biosynthesis of specific proteins related to biologic response of that steroid (44, 45). The extraordinary rapidity of onset of response observed renders the accepted mechanism of action of steroid hormone highly unlikely in these experiments.

Despite the uncertainties concerning effective doses, our observations suggest important clinical implications. For example, it is possible that, in the genesis of hyperparathyroidism, the problem resides less in the continued stimulation of PTH secretion by some known or unknown secretogogues, but rather in the failure of dampening signals to become effective. The experiments also suggest some therapeutic possibilities because 24,25-$(OH)_2D_3$, with its weak vitamin D-like actions, may represent an effective suppressor of PTH secretion in patients with hyperparathyroidism who are not candidates for surgical cure.

An interpretive model for these data can be constructed by combining data in the literature with our present observations. 24,25-$(OH)_2D_3$ is a major metabolite of vitamin D_3 of unknown biological significance. Its concentration in normal peripheral plasma may reach hundreds of times that of 1,25-$(OH)_2D_3$. Several lines of evidence show that the 24-hydroxylase of 25-$(OH)D_3$ is induced by the presence of 1,25-$(OH)_2D_3$ (46, 47). This enzyme acts indiscriminately on both 25-$(OH)D_3$ and 1,25-$(OH)_2D_3$. The role of the trihydroxy vitamin D_3 (1,24,25-$[OH]_3D_3$) in calcium homeostasis is totally unknown and its effect on PTH secretion has not been tested. In view of this information, the PTH-D_3 feedback loop may be as follows: PTH → stimulation of 1,25-$(OH)_2D_3$ formation → stimulation of 24-hydroxylase of 25-$(OH)D_3$ → increased circulating concentration of 24,25-$(OH)_2D_3$ → suppression of PTH secretion. With the development of assays for 24,25-$(OH)_2D_3$, this hypothesis can be tested.

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