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Control of Kidney 25-Hydroxyvitamin D_3 Metabolism

Strontium and the Involvement of Parathyroid Hormone[1]

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The action of parathyroid extract (PTE) on the renal metabolism of 25-hydroxyvitamin D_3 (25-OHD_3) was evaluated in rat models for strontium rickets and hypoparathyroidism. PTE elevated the production of 1α,25-$(OH)_2D_3$ and suppressed the synthesis of 24,25-$(OH)_2D_3$ in both animal models. Part of strontium's action in suppressing 1α,25-$(OH)_2D_3$ and stimulating 24,25-$(OH)_2D_3$ synthesis in strontium rickets appears to involve a decrease in parathyroid hormone (PTH) secretion and/or action. Calcitonin (CT) was not implicated in the cation's action. Thyroparathyroidectomized rats showed a low level of 1α,25-$(OH)_2D_3$ production which increased four- to eightfold following chronic PTE treatment. PTH appears to be the major calcium regulatory hormone involved in modulation of renal 25-OHD_3 metabolism.

Kidney metabolism of 25-hydroxyvitamin D_3 (25-OHD_3)[2] can be modulated by altering the ambient concentration of certain calcium homeostatic hormones (1–5) and ions (6–9). Parathyroid hormone (PTH) (1, 3, 4), low dietary calcium (6), and low dietary phosphorus (7, 8) are purported to stimulate mitochondrial 25-hydroxyvitamin D_3 1α-hydroxylase (25-OHD_3-1α-OH'ase) activity while ablation of the hormone (1, 3, 5) or feeding of diets high in calcium (6, 9) effectively suppresses the 1α-hydroxylase enzyme and induces 25-hydroxyvitamin D_3 24-hydroxylase (25-OHD_3-24-OH'ase) enzyme activity.

Since PTH secretion is controlled mainly by serum ionized-calcium concentration, it is conceivable that PTH could function as a common effector of the observed ion-prompted changes in 25-OHD_3 metabolism. However, thyroparathyroidectomized (TPTX) rats fed a low-phosphorus high-calcium diet are able to maintain 1α-hydroxylase activity in the absence of PTH (7, 12). From these and other studies it has been suggested that phosphorus (7, 8) and/or calcium (13) are involved in the intracellular induction and suppression of 25-OHD_3-1α-OH'ase and 25-OHD_3-24-OH'ase activities. Consequently, the subject concerning a strict PTH dependence for the expression of kidney 25-OHD_3-1α-OH'ase activity is currently equivocal (1, 3, 10, 11).

Animals are sensitive to their calcium

[1] This is paper No. 2 in the series. Paper No. 3 (J. L. Omdahl and A. P. Evan, 1977, *Arch. Biochem. Biophys.* 184, 179–188) directly follows.

[2] Abbreviations used: 25-OHD_3, 25-hydroxyvitamin D_3; 25-OHD_3-1α-OH'ase, 25-hydroxyvitamin D_3 1α-hydroxylase; 25-OHD_3-24-OH'ase, 25-hydroxyvitamin D_3 24-hydroxylase; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25-OH[^{3}H]D_3, 25-[26,27-^{3}H]hydroxyvitamin D_3; PTH, parathyroid hormone; CT, calcitonin; PTE, parathyroid extract; PTX, parathyroidectomized; TPTX, thyroparathyroidectomized; c-AMP, adenosine 3':5'-cyclic phosphate; c-GMP, guanidine 3':5'-cyclic phosphate; iv, intravenous; ip, intraperitoneal; (−)strontium, low-calcium/low-phosphorus diet (25 mmol/kg of calcium, 0.1% Ca; 32 mmol/kg of phosphorus, 0.1% P); (+) strontium, (−)strontium diet supplemented with 90 mmol/kg of strontium (0.79% Sr); (+)calcium, (−) strontium diet supplemented with 90 mmol/kg of calcium (0.36% Ca).

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balance as evidenced by an inverse relationship between dietary calcium intake and 1α-hydroxylase activity (9). An increase in 25-OHD_3-1α-OH'ase activity due to a low calcium intake results in stimulated synthesis of 1α,25-$(OH)_2D_3$, a dihydroxy metabolite which is extremely active in stimulating intestinal calcium absorption and bone mineral mobilization (14). However, such a response to a low calcium intake can be negated if strontium is supplied in the diet. In fact, strontium inhibits intestinal calcium transport by suppressing 1α-hydroxylase activity (15) and consequently the production of 1α,25-$(OH)_2D_3$, thereby facilitating diminution of bone mineralization and development of strontium rickets. Such results suggest that strontium effects a lesion in the hormonal and/or end-organ components of the control loop whereby 25-OHD_3-1α-OH'ase activity is modulated in response to the organism's calcium need. Therefore, in an attempt to more clearly define the control of 25-OHD_3 metabolism, we elected to use the strontium-rachitic model as a means of evaluating the control of renal 25-OHD_3 metabolism by PTH and calcium. Partial mediation of strontium's action through an alteration in peripheral PTH titer is addressed in the present communication.

MATERIALS AND METHODS

Animals. Holtzman (Madison, Wisconsin) weanling male rats were housed in hanging wire cages, given distilled water, and fed *ad libitum* a vitamin D-deficient diet (16) (0.4% DL-methionine used instead of cysteine) for 3–4 weeks prior to experimental use. During the experimental period a low-calcium/low-phosphorus diet (25 mmol/kg of calcium, 0.1% Ca; 32 mmol/kg of phosphorus, 0.1% P), designated (−)strontium, was used directly or supplemented (90 mmol/kg) with strontium (0.79% Sr) or calcium (0.36% Ca). The latter diets are designated (+)strontium and (+)calcium, respectively, and were fed for 11 days as specified in the text.

In vivo 25-OH[^{3}H]D_3 metabolism. Radioactive 25-hydroxyvitamin D_3 (25-OH[26,27-^{3}H]D_3; 6–9 Ci/mmol) was purchased from Amersham/Searle (Arlington Heights, Illinois) and purified by column chromatography using Sephadex LH-20 (hexane:chloroform, 1:1) (17). Rats were given intravenous (iv) injections (30 μl; ethanol:water, 95:5) of 25-OH[26,27-^{3}H]D_3 (7×10^5 dpm, 25-OH[^{3}H]D_3) 12 h prior to sacrifice. Serum and intestinal samples were mixed with [^{14}C]cholesterol (3×10^3 dpm) for estimation of sample recovery and subsequently extracted using methanol-chloroform (18) with the radioactive metabolic products separated using Sephadex LH-20 (chloroform:hexane, 7:3, 90 ml) column chromatography (1.4 × 20 cm; 2.5-ml fractions) (17, 19). Periodate cleavage of vicinal hydroxyl groups was used to separate 1α,25-OH_2[^{3}H]D_3 from 25,26-$(OH)_2$[^{3}H]D_3 (20).

Urinary cyclic AMP and cyclic GMP. Rats were maintained in individual metabolic cages with urine collections taken every 24 h during the experimental period. Rats were fed the (−)strontium diet for 5 days. The experimental period was started by splitting the animals into three dietary groups: (i) (−)strontium, (ii) (+)strontium, and (iii) (+)calcium. These diets were fed for 8 days, at which time urine analysis for cyclic AMP and cyclic GMP was initiated and continued for a 3-day period (Days 9–11). The cyclic nucleotides were measured by radioimmunoassay (21), with the results expressed as picomoles per milligram of creatinine.

Intestinal ^{45}Ca transport. The proximal 5 cm of duodenal tissue was everted by using a glass rod, blotted free of intestinal materials, and used for the estimation of mucosal to serosal ^{45}Ca transport as previously described (22).

Data reduction and analysis. Radioactivity of the separated metabolites was determined by liquid scintillation spectrophotometry (Beckman LS-245) in which the data were reduced and stored on punch tape. Data were analyzed by using a Hewlett-Packard model 9830 computer to plot the chromatographic profile and calculate peak areas. Final results were expressed as disintegrations per minute of metabolite.

Thyroparathyroidectomy. Rats fed the vitamin D-deficient diet for 3–4 weeks were given 625 pmol of 25-OHD_3 [intraperitoneal (ip), 50 μl of propylene glycol:ethanol, 7:3] and placed on the (−)strontium diet 2 days before thyroparathyroidectomy. The thyroid and parathyroid glands were removed by blunt dissection (ether anesthesia) followed by an (ip) injection of parathyroid extract (5 IU, Eli Lilly) at 5 h postsurgery. A peripheral blood sample was taken 24 h after removal of the glands to document surgical success. A decrease in total serum calcium of 2.5 mg/dl (24 h after surgery) was used as an index for successful thyroparathyroidectomy. Thyroxine replacement was given ip (5 μg every 48 h). Sham control rats were prepared by manipulating but not removing the thyroid and parathyroid glands.

General chemical procedures. Total serum calcium and strontium concentrations were determined by atomic absorption spectrophotometry (Instrumentation Laboratory, Model 251) (19). Protein was measured by using a modified Lowry technique (23) and phosphorus was determined by the method of Chen *et al.* (24).

RESULTS

The hypothesis that strontium acts to suppress parathyroid hormone (PTH) secretion (25) or stimulate calcitonin (CT) release (26, 27), resulting in decreased renal $1\alpha,25\text{-}(OH)_2D_3$ production and increased $24,25\text{-}(OH)_2D_3$ synthesis, was addressed in the current study. Such a possibility regarding decreased PTH secretion was evaluated by using the urine adenosine 3′:5′-cyclic phosphate (c-AMP) concentration as an indirect index of PTH secretory activity, due to the hormones well-documented action to stimulate renal adenyl cyclase activity (28). A significant decrease in urine c-AMP ($P < 0.01$) was observed for both (+)strontium- and (+)calcium-fed rats, although calcium appeared more effective than strontium ($P < 0.05$) (Table I). It is suggested from such results that strontium may effect an *in vivo* pseudocalcemic action resulting in decreased secretion and/or trophic renal function for PTH. Urine guanidine 3′:5′-cyclic phosphate (c-GMP) concentration, in contrast to c-AMP, did not vary with dietary treatment. PTH's involvement as a mediator of strontium's action was verified by evaluating the cation's *in vivo* action in rats supplemented with parathyroid extract (PTE). Rats fed a (+)strontium diet and simultaneously treated with PTE showed an increase in serum and intestinal $1\alpha,25\text{-}(OH)_2[^3H]D_3$ content and a depressed serum level of $24,25\text{-}(OH)_2\text{-}[^3H]D_3$ (Table II). It is evident from these studies that strontium's action is accomplished, at least in part, by a suppression of the secretion of PTH.

Consideration was next given to the

TABLE I

DIETARY STRONTIUM AND CALCIUM INFLUENCES ON URINE CONCENTRATION OF CYCLIC AMP AND CYCLIC GMP[a]

Dietary treatment	Urine concentration[b] (pmol/mg of creatinine)	
	c-AMP	c-GMP
(−)Strontium	39.3 ± 10.8	6.6 ± 2.2
(+)Strontium	19.1 ± 2.5[c]	7.8 ± 1.6
(+)Calcium	10.9 ± 6.0[c, d]	6.8 ± 1.9

[a] Rats were fed the respective diets for 8 days, with urine collected on Days 9–11 for c-AMP and c-GMP determinations. See Materials and Methods for details.

[b] Values are 3-day means ± SD for four animals.

[c] Significantly different from (−)strontium group ($P < 0.01$, unpaired t test).

[d] Significantly different from (+)strontium group ($P < 0.05$).

TABLE II

THE INFLUENCE OF PARATHYROID EXTRACT ON SERUM AND INTESTINAL $24,25\text{-}(OH)_2[^3H]D_3$ AND $1\alpha,25\text{-}(OH)_2[^3H]D_3$ CONCENTRATIONS IN (+)STRONTIUM RATS[a]

Group	$24,25\text{-}(OH)_2[^3H]D_3$	$1\alpha,25\text{-}(OH)_2[^3H]D_3$
	Serum[b] (dpm/ml)	
Sham	3642 ± 730	179 ± 309
Sham + 20 U[c]	1150 ± 566[c]	1804 ± 1026[d]
TPTX	6614 ± 2760	0
TPTX + 20 U	2242 ± 618	760 ± 240[d]
	Intestine[b] (dpm/g)	
Sham	–	197 ± 334
Sham + 20 U	–	1682 ± 1206[c]
TPTX	–	0
TPTX + 20 U	–	800 ± 170[d]

[a] Rats were fed the (+)strontium diet for 11 days and were subsequently given an iv injection of $25\text{-}OH[^3H]D_3$ (7×10^5 dpm); serum and total intestinal radioactivities were collected 12 h later. See Materials and Methods for further details.

[b] Data are given as mean ± SD for four animals.

[c] Parathyroid extract (20 units) or carrier (1.6% glycerine) was given every 8 h (ip) for 11 days to sham or thyroparathyroidectomized (TPTX) rats.

[d] Significantly different from matched carrier group ($P < 0.01$).

[c] Significantly different from matched carrier group ($P < 0.05$).

possibility that strontium's action may, in addition, involve a concomitant stimulation in the release of calcitonin (CT) (26, 27). Thyroparathyroidectomized (TPTX) rats were used in conjunction with PTE supplementation to study the possible PTH and CT actions and interdependencies. If CT was a major modulating factor for strontium's action in suppressing 1α-hydroxylase and inducing 24-hydroxylase activities, then removal of the thyroid gland should negate the hormone's action. However, (+)strontium-TPTX rats did not show an increase in 1α,25-$(OH)_2D_3$ synthesis. Removal of the thyroparathyroid axis resulted in a further lowering of serum and intestinal 1α,25-$(OH)_2[^3H]D_3$ levels and an increase in serum 24,25-$(OH)_2[^3H]D_3$ (Table II). In contrast, PTE supplementation of the (+)strontium-TPTX rats prompted an increase in serum and intestinal 1α,25-$(OH)_2[^3H]D_3$ and a decrease in serum 24,25-$(OH)_2[^3H]D_3$, similar to results obtained in sham animals. This action of PTE was not influenced by CT (i.e., similar PTE response in sham and TPTX animals), which suggests that PTH is the dominant calcium-regulating hormone regarding modulation of kidney 25-OHD_3 metabolism.

The intestinal target-organ sequestration of 1α,25-$(OH)_2D_3$ was observed throughout the study to be a valid index of the metabolite's stimulation of intestinal calcium absorption ($r = 0.99$). A low level of calcium transport activity (equivalent to the vitamin D-deficient response) was detected in (+)strontium- compared to (−)strontium-fed rats, due to the previously mentioned action of the cation to suppress renal 25-OHD_3-1α-OH'ase activity. Production of 1α,25-$(OH)_2D_3$ in (+)strontium animals increased in response to PTE treatment (Table III), resulting in a concomitant elevation in intestinal calcium transport (Fig. 1). Collectively, these results substantiate one aspect of strontium's inhibitory action on intestinal calcium transport as a lesion in the PTH-directed renal synthesis of 1α,25-$(OH)_2D_3$. Aside from PTE's action on renal 25-OHD_3 hydroxylation, the extract was also physiologically active in elevating serum calcium and lowering serum phosphorus. PTE effected an equivalent elevation in serum calcium for rats in both the (+)-strontium and (−)strontium groups. Serum phosphorus was consistently lower in the (+)strontium compared to (−)strontium group, although both groups responded to PTE with a lowering of serum phosphorus (Table IV). The difference in serum phosphorus concentrations between groups may be attributable to strontium's

TABLE III

PARATHYROID EXTRACT EFFECTS ON INTESTINAL 1α,25-$(OH)_2[^3H]D_3$ SEQUESTRATION IN (+)STRONTIUM AND (−)STRONTIUM RATS[a]

	Group	1α,25-$(OH)_2[^3H]D_3$ (dpm/g)	25-OHD concentration (pmol/ml)
	Experiment I		
(+)Strontium	Sham	193 ± 334[b]	29.0 ± 11
	TPTX	0	47.3 ± 7.3
	TPTX + 8 U[c]	444 ± 180[d]	21.8 ± 0.3
	TPTX + 20 U	800 ± 170[d]	37.3 ± 19.5
	Experiment II		
(−)Strontium	Sham	1125 ± 50[c]	31.3 ± 8.8
	TPTX	364 ± 100	40.3 ± 10.3
	TPTX + 8 U	1560 ± 563[d]	43.5 ± 4.3
	TPTX + 20 U	2690 ± 975[d]	23.5 ± 3.5

[a] Experimental protocol was the same as that described in Table II except that both (+)strontium and (−)strontium diets were used. See Materials and Methods for details.

[b] Values are the mean ± SD for three to four animals.

[c] Parathyroid extract (8 or 20 units) or carrier (1.6% glycerine) was given every 8 h (ip) for 11 days.

[d] Significantly different from TPTX group ($P < 0.01$).

pseudocalcemic action, which results in potentiation of the inverse physicochemical relationship between serum calcium and phosphorus.

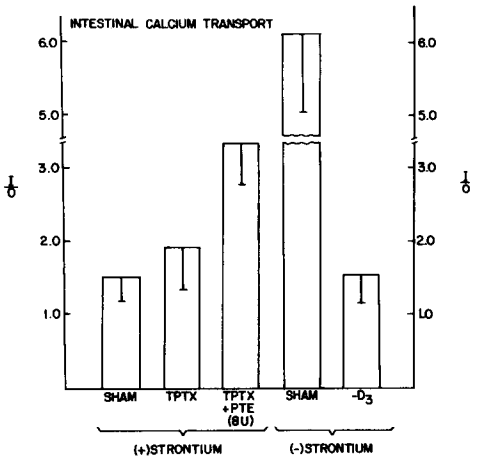

FIG. 1. Inverted gut sac measurement of intestinal calcium transport. Rats were fed the respective diets (see Materials and Methods) and given ip injections of PTE (8 U/8 h) or carrier (1.6% glycerin) for 11 days following verification of surgical TPTX. The two (−)strontium groups differ only in 25-OHD_3 repletion or deficiency states and give the extremes for the range of calcium transport activities. The (−)strontium sham group shows adaptation to a low-calcium diet (high activity), whereas the $-D_3$ group demonstrates the response seen in vitamin D deficiency (low activity). Values are expressed as the ratio of ^{45}Ca inside to ^{45}Ca outside the gut sac preparation and represent the mean ± SD for five animals.

DISCUSSION

Animals with strontium rickets are characterized by having decreased kidney 25-OHD_3 1α-hydroxylase and increased 25-OHD_3 24-hydroxylase enzyme activities (15, 29). Such an action on the 1α-hydroxylase enzyme mimics what is observed in animal models for acute (1, 3, 5) and chronic hypoparathyroidism (30). Since strontium is capable of directly suppressing PTH secretion (25), it was postulated that the cation could indirectly alter 1α-hydroxylase and 24-hydroxylase enzyme activities by decreasing peripheral PTH concentration. This study substantiates such a postulate wherein parathyroid extract (PTE) treatment of strontium-fed rats resulted in a stimulation of 1α,25-$(OH)_2D_3$ and a decrease in 24,25-$(OH)_2D_3$ production. Although strontium is also known to stimulate calcitonin (CT) release (26, 27), it does not appear that CT is involved in strontium's action. Rather, PTH appears to be the dominant calcium-regulating hormone with regard to modulation of kidney 25-OHD_3 metabolism.

Aside from strontium's association with PTH secretion, it appears that the cation's action on 25-OHD_3 metabolism may also involve other factors. Exemplary is the

TABLE IV

SERUM CALCIUM, STRONTIUM, AND PHOSPHORUS CONCENTRATIONS IN (+)STRONTIUM AND (−)STRONTIUM RATS[a]

Treatment	Group	Ion concentrations[b] (mg/100 ml)		
		Ca	Sr	P
(+)Strontium	Sham	5.8 ± 0.3[c]	7.2 ± 1.1[d]	8.3 ± 1.0[d]
	TPTX	4.6 ± 1.0	4.5 ± 1.0	11.3 ± 1.4
	TPTX + PTE (8 U)[e]	7.2 ± 0.6[d]	7.0 ± 1.7[c]	7.3 ± 1.0[d]
	TPTX + PTE (20 U)	6.9 ± 0.6[d]	7.4 ± 0.3[d]	7.7 ± 0.8[d]
(−)Strontium	Sham	9.4 ± 0.5[d]	–	9.2 ± 0.4[d]
	TPTX	4.5 ± 0.6	–	14.1 ± 1.2
	TPTX + PTE (8 U)	6.8 ± 2.5	–	9.9 ± 0.7[d]
	TPTX + PTE (20 U)	7.5 ± 0.9[d]	–	8.3 ± 2.6[d]

[a] Experiment Protocol was the same as that described in Table III.
[b] Values are the mean ± SD for four to six animals.
[c] Significantly different from TPTX group ($P < 0.05$).
[d] Significantly different from TPTX group ($P < 0.01$).
[e] See legend to Table III.

observation that PTE's stimulation of $1\alpha,25\text{-}(OH)_2D_3$ production in (+)strontium rats was only ~35% of that observed in animals not given strontium [i.e., (−)strontium, Table III]. This observation could be attributed to various factors such as altered metabolism and turnover of PTE and/or $1\alpha,25\text{-}(OH)_2D_3$, a strontium antagonism of PTE's renal-trophic action, or a direct antagonism at the enzyme level by strontium. Although the involved factors have not been studied directly, it is evident from the current study that PTE's physiological action to raise serum calcium and lower serum phosphorus is not impeded by dietary strontium treatment (Table IV), suggesting that PTE's renal-trophic action may be unaltered.

From studies to date, it appears that strontium rickets are developed, in part, due to dietary strontium's interference with PTH secretion and/or action, resulting in diminished $1\alpha,25\text{-}(OH)_2D_3$ synthesis and subsequent lowering of intestinal calcium absorption. Development of rachitic bone lesions could then be attributed to the combined effects of an inhibition in intestinal calcium absorption and a direct action by strontium to inhibit the calcium-dependent bone-mineralization process.

In addition to PTH's implications in strontium rickets, the present study also addresses the topic of PTH's action on kidney $25\text{-}OHD_3$ metabolism in chronic hypoparathyroid rats. At present, the direct demonstration of an action for PTH in promoting $1\alpha,25\text{-}(OH)_2D_3$ synthesis has been accomplished only in acute parathyroidectomized (PTX) (3) or TPTX animals (1). The more physiologically relevant chronic action of the hormone has not been thoroughly investigated. Yet, it is evident from this study that PTE markedly stimulates $1\alpha,25\text{-}(OH)_2D_3$ production in chronic TPTX rats fed a diet low in both calcium and phosphorus (Table III). Although some $1\alpha,25\text{-}(OH)_2D_3$ synthesis occurs in TPTX rats, the amount appears insignificant when compared to the metabolite's production in sham or PTE-supplemented animals. As previously described, 1α-hydroxylase activity is maintained in TPTX rats fed a high-calcium/low-phosphorus diet (7, 12), which raises the question of whether PTH should be called a kidney-trophic agent concerning its influence on 25-hydroxyvitamin D metabolism. This topic remains a moot point. However, it is evident that PTH is a significant physiological regulator of ambient $1\alpha,25\text{-}(OH)_2D_3$ concentration (30), where the hormone stimulates $1\alpha,25\text{-}(OH)_2D_3$ production in low-phosphorus-fed chronic TPTX rats which have either low (present communication) or high (personal observation) calcium intakes.

How PTH prompts such an intracellular action is currently unresolved. Factors involved in mediating the hormone's action may include c-AMP (28), calcium (13), phosphorus (7), and/or $1\alpha,25\text{-}(OH)_2D_3$ (20, 31). The extent to which PTH interacts with one or more of these factors and the possibility that the resultant change in hydroxylase enzyme specificity requires *de novo* protein synthesis are presently unclear and the topics of current investigations.

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