Evidence That Blood Ionized Calcium Can Regulate Serum $1,25(OH)_2D_3$ Independently of Parathyroid Hormone and Phosphorus in the Rat

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Abstract

This study asks whether arterial blood ionized calcium concentration (Ca^{++}) can regulate the serum level of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] independently of serum phosphorus and parathyroid hormone (PTH). We infused either PTH (bovine 1-34, 10 U/kg body wt/h) or saline into awake and unrestrained rats for 24 h, through a chronic indwelling catheter. PTH raised total serum calcium and arterial blood ionized calcium, yet serum $1,25(OH)_2D_3$ fell from 35±6 (mean±SEM, n = 10) with saline to 12±3 pg/ml (n = 11, $P < 0.005$ vs. saline). To determine if the decrease in serum $1,25(OH)_2D_3$ was due to the elevated Ca^{++}, we infused PTH into other rats for 24 h, along with varying amounts of EGTA. Infusion of PTH + 0.67 μm/min EGTA reduced Ca^{++}, and $1,25(OH)_2D_3$ rose to 90±33 ($P < 0.02$ vs. PTH alone). PTH + 1.00 μm/min EGTA lowered Ca^{++} more, and $1,25(OH)_2D_3$ increased to 148±29 ($P < 0.01$ vs. saline or PTH alone). PTH + 1.33 μm/min EGTA lowered Ca^{++} below values seen with saline or PTH alone, and $1,25(OH)_2D_3$ rose to 267±46 ($P < 0.003$ vs. all other groups). Thus, during PTH infusion lowering Ca^{++} with EGTA raised $1,25(OH)_2D_3$ progressively. There were no differences in serum phosphorus concentration or in arterial blood pH in any group infused with PTH. The log of serum $1,25(OH)_2D_3$ was correlated inversely with Ca^{++} in all four groups infused with PTH ($r = -0.737$, n = 31, $P < 0.001$), and also when the saline group was included ($r = -0.677$, n = 41, $P < 0.001$). The results of this study indicate that serum $1,25(OH)_2D_3$ may be regulated by Ca^{++} independent of PTH and serum phosphorus levels in the rat. Since $1,25(OH)_2D_3$ regulates gastrointestinal calcium absorption, there may be direct feedback control of $1,25(OH)_2D_3$, by its regulated ion, Ca^{++}.

Introduction

It is difficult to tell if blood ionized calcium concentration (Ca^{++})[1] directly regulates the production and serum level of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. Because increased Ca^{++} downregulates secretion of parathyroid hormone (PTH), and PTH can stimulate $1,25(OH)_2D_3$ production directly (1–4) and by lowering blood phosphorus concentration (5, 6), any change in Ca^{++} that alters PTH can affect $1,25(OH)_2D_3$ via PTH. Consequently, direct effects of Ca^{++} itself upon serum $1,25(OH)_2D_3$ are difficult to isolate. Parathyroidectomy, an obvious way to disentangle the effects of Ca^{++} and PTH, produces hyperphosphatemia and hypocalcemia that complicate the interpretation of experimental results. For example, low calcium diet raises serum $1,25(OH)_2D_3$ (5, 7–12) and PTH (5, 7, 10–12) in rats; in some experiments $1,25(OH)_2D_3$ increases despite parathyroidectomy (11, 13, 14) but in others it does not (5, 15). At best, the response of $1,25(OH)_2D_3$ to low calcium diet is much below normal after parathyroidectomy (11). Whether the variable and submaximal response of serum $1,25(OH)_2D_3$ to low calcium diet is due to hyperphosphatemia, lack of PTH, or the fact that Ca^{++} truly can regulate serum $1,25(OH)_2D_3$ only weakly, is not clear. Chronic metabolic acidosis raised blood Ca^{++} and lowered $1,25(OH)_2D_3$ in rats eating a low calcium diet even though their PTH levels, judged by radioimmunoassay, seemed the same as in nonacidotic rats eating the same diet (12). However, small changes in PTH that the assay cannot register, may affect $1,25(OH)_2D_3$ production, so the experiment is not a critical test of the hypothesis that Ca^{++} directly regulates $1,25(OH)_2D_3$.

It is worthwhile to look for a direct effect of Ca^{++} on $1,25(OH)_2D_3$ in vivo, because medium Ca^{++} can regulate $1,25(OH)_2D_3$ production in vitro. Conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ by kidney homogenates (16, 17), isolated tubules (18), and kidney cells (19) is inhibited by increased medium calcium. Incidentally, mitochondrial conversion is promoted by increased Ca^{++} (20), suggesting a difference between calcium effects on cells and mitochondria.

Our strategy in the present study was to infuse large amounts of PTH for 24 h, to minimize the influence of endogenous PTH secretion on $1,25(OH)_2D_3$ production, to stabilize serum phosphorus by providing an ample dietary phosphorus supply, and to lower arterial blood Ca^{++} by infusing EGTA. The question was whether Ca^{++} can regulate serum $1,25(OH)_2D_3$ levels despite a constant, high PTH infusion and stable serum phosphorus levels; our result was that it can.

Methods

Animals

We placed chronic arterial and venous catheters in 250–275 g adult male Sherman rats (Camm Research Lab Animals, Wayne, NJ) under light hexobarbitol anesthesia (12, 21). After surgery, we placed each rat in its own metabolic cage and connected the catheters, encased in a stainless steel spring, to a swivel device (Instech Laboratories, Inc., Fort Washington, PA) that permitted free movement within the cage. Rats ate chow containing 1.2% calcium, 0.99% phosphorus, and 2.2 IU of vitamin D_2/g food, and drank deionized distilled water ad libitum. After 11 d we

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1. *Abbreviations used in this paper:* $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; Ca^{++}, arterial blood ionized calcium concentration; HPLC, high performance liquid chromatography; TPTX, thyroparathyroidectomized; PTE, parathyroid extract; PTH, parathyroid hormone.

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Table I. Response of Neonatal Mouse Calvariae to PTH

Group	n	ICa	FCa	JCa	IpH	$IPco_2$	$IHCO_3$
		mg/dl	*mg/dl*	*nmol/bone/24 h*	*nmol/bone/24 h*	*mmHg*	*meq/liter*
Control	14	7.14±0.06	7.39±0.07	57±16	7.39±0.01	37.9±0.3	22.9±0.3
PTH	5	7.07±0.04	8.39±0.10*	297±18*	7.38±0.01	37.1±0.4	22.0±0.6

Abbreviations: Ca, calcium; F, final medium; HCO_3, bicarbonate; I, initial medium; J, net flux, calculated as (FCa − ICa) × medium volume/two bones; n, number of calvarial pairs in each group; Pco_2, partial pressure of carbon dioxide. PTH, calvariae cultured in 1×10^{-8} M synthetic bovine 1–34 parathyroid hormone for 24 h. * Different than control, $P < 0.001$.

divided the rats randomly into two groups for protocol 1, or into three groups for protocol 2.

Experimental design

Protocol 1. For 24 h, each rat received through the venous catheter 75 mM NaCl at 0.4 ml/h either alone (saline group) or with synthetic bovine parathyroid hormone 1–34 (PTH group) at a concentration sufficient to deliver 10 U/kg body weight/h (PTH from Beckman Instruments, Inc., Fullerton, CA). This dose of PTH provides slightly over three times the basal replacement dose of bovine parathyroid hormone in thyroparathyroidectomized rats as estimated by Takahashi et al. (22). This large dose of PTH was chosen to provide maximal hormonal stimulation to the proximal tubule cells as judged by calcium transport characteristics (23) and, thereby, to minimize the effects of endogenous PTH secretion.[2]

Protocol 2. Each rat received PTH in saline exactly as in protocol 1 but the infusion also contained enough EGTA to deliver 0.67 μm/min (PTH + 0.67 EGTA group), 1.00 μm/min (PTH + 1.00 EGTA group), or 1.33 μm/min (PTH + 1.33 EGTA group). We used EGTA (Sigma Chemical Co., St. Louis, MO) to clamp the level of ionized calcium rather than EDTA (24) because of its lower affinity for magnesium (25).

Infusions and collections. We adjusted all solutions to pH 7.40 with concentrated HCl or NaOH before infusion. At the initiation of each 24-h infusion, 0.4 ml of the infusate was given over the first 3 min. From the fourth day after surgery we measured food and fluid intake daily in all rats and eliminated those rats that ate less than 13 g of food or drank less than 15 ml of fluid on any day. We measured calcium, creatinine, phosphorus, and cyclic AMP in the urine that was collected (in 0.25 ml of 12 N HCl) during the 24-h infusion. At the end of the infusion we withdrew blood from the arterial catheter without anesthesia and then sacrificed the rats. Some whole blood was used for acid-base and Ca^{++} measurements. From the rest of the blood we separated serum from cells within 30 min, and froze a portion of it at −25°C for $1,25(OH)_2D_3$ measurements. The rest of the serum was used for the remainder of the measurements. Each rat was infused only once.

Biological activity of PTH

To verify the biological activity of the infused PTH we tested its ability to enhance calcium resorption from neonatal mouse calvariae. 4–6-d old Swiss Cox mice were killed and their calvariae were removed by dissection (26, 27). Exactly 2.8 ml of culture medium (Dulbecco's modified Eagles medium with 4.5 g/liter glucose, heat-inactivated [1 h at 56°C] horse serum [15%], and sodium heparin [10 U/ml]) at pH between 7.35 and 7.45 (adjusted with concentrated HCl or NaOH) was preincubated at 37°C, Pco_2 40 mmHg, for 3 h in 35-mm Petri dishes. 1 ml was then removed to determine preincubation medium pH, Pco_2, and calcium concentration, and two calvariae were placed in the dish on a stainless steel wire grid. Bones were cultured for 24 h, at 37°C, with or without 10^{-8} M PTH and then a second sample of culture medium was obtained and similarly analyzed.

2. To the best of our knowledge no studies exist that relate PTH infusion to production of $1,25(OH)_2D_3$ in vivo, in rat proximal tubule cells, from which we could derive that infusion rate which provides maximal hormonal stimulation to $1,25(OH)_2D_3$ production.

Calvariae incubated with PTH raised medium calcium by 1.32±0.08 mg/dl vs. 0.25±0.07 in control bones ($P < 0.001$). Net calcium flux, calculated as the final calcium concentration minus the initial calcium concentration times the volume of culture medium divided by two bones, was higher with PTH incubation (297±18 nmol/bone/24 h vs. 57±16, PTH vs. control, $P < 0.001$, Table I). The increases in medium calcium and calcium flux with PTH are similar to what we (26, 27) and others (28) have found previously, and indicate that the hormone was biologically active. There was no difference in initial medium calcium, pH, Pco_2, or bicarbonate between bone cultures with or without PTH (Table I).

$1,25(OH)_2D_3$ measurements

We thawed 1–2 ml of serum from each rat at room temperature, and added 1,500 cpm of $[^3H]1,25(OH)_2D_3$ (specific activity, 91 Ci/mmol; Amersham Corp., Arlington Heights, IL) to monitor procedural losses. Lipid-soluble vitamin D metabolites were extracted from serum with methylene chloride/methanol (1:1) and washed twice with phosphate buffer (pH = 10.4). $1,25(OH)_2D_3$ was separated from other metabolites and contaminants by Sephadex LH 20 gravity flow chromatography, and then by high performance liquid chromatography (HPLC) using previously described solvent systems (10, 12, 21, 29, 30). The HPLC effluent containing $1,25(OH)_2D_3$ was assayed in triplicate in a competitive binding radioreceptor assay that uses the 100,000 g supernatant of homogenized vitamin D-deficient chick intestinal epithelium as the source of the $1,25(OH)_2D_3$ receptor (31). Sensitivity of the assay ranged from 2 to 7 pg/assay tube. Interassay coefficient of variation between the five assays performed was 19.6%. Overall sample recovery was 55±5%.

Other laboratory measurements

We measured arterial blood ionized calcium using a micro-electrode (Orion Biomedical Space Stat 20; Orion Research Inc., Cambridge, MA [12]), and total calcium in serum and urine by atomic absorption spectrophotometry (Instrumentation Laboratory, Lexington, MA) using aqueous standards (12, 29, 30). We measured creatinine in urine and phosphorus in serum and urine using an Autoanalyzer (Model AA1; Technicon Instruments Corp., Tarrytown, NY), serum creatinine by the Heinegard and Tiderstrom modification of the Jaffe reaction, and urinary cyclic AMP using radioimmunoassay (New England Nuclear, Boston, MA) (12, 29, 30). We measured arterial blood pH and Pco_2 using a pH-blood gas analyzer (Radiometer BMS Mk3, Copenhagen, Denmark). We calculated plasma bicarbonate concentration from the pH and Pco_2 using the Henderson-Hasselbalch equation with a solubility coefficient of 0.0306 and pK of 6.099 (at pH 7.40) that was corrected for pH (12, 21, 26, 27).

Statistical methods

We assessed differences between groups using t tests that did not assume equal variances in the groups being compared. Regressions were calculated by least squares. The t tests and regressions were calculated using standard digital computer methods (BMDP, University of California at Los Angeles). Mean values are ±SE; ns, indicates nonsignificance, $P \geq 0.05$.

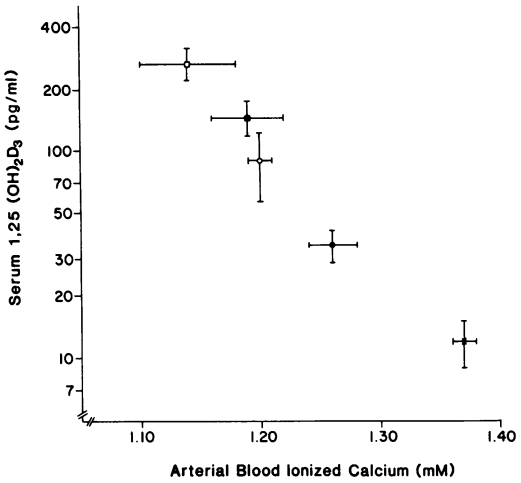

Figure 1. Effect of arterial blood ionized calcium (Ca^{++}) on serum $1,25(OH)_2D_3$. Values are mean±SE. Rats were infused (see Methods) for 24 h with NaCl alone (closed circle, n = 10) at 0.4 ml/h; 10 μm/kg per h of synthetic bovine PTH 1–34 (x, n = 11); PTH and 0.67 μm/min EGTA (open circle, n = 7); PTH and 1.00 μm/min EGTA (closed square, n = 6); or PTH and 1.33 μm/min EGTA (open square, n = 7). Log serum $1,25(OH)_2D_3$ was correlated inversely, for all data included, with Ca^{++} ($r = -0.677$, $n = 41$, $P < 0.001$).

Results

Ionized calcium, phosphorus, and $1,25(OH)_2D_3$. Infusion of PTH increased arterial Ca^{++} and lowered serum $1,25(OH)_2D_3$ (Fig. 1, × symbol) compared with saline alone (Fig. 1, closed circle). Serum $1,25(OH)_2D_3$ fell in response to PTH infusion, even though the PTH raised both total calcium level and Ca^{++}, and lowered serum phosphorus level (Table I; and Fig. 1).

To test the idea that increased Ca^{++} itself had suppressed serum $1,25(OH)_2D_3$ despite PTH excess, we infused the same amount of PTH with three different concentrations of EGTA, to lower Ca^{++}. As Ca^{++} was lowered progressively, serum $1,25(OH)_2D_3$ rose (Fig. 1, open circle, closed square, and open square; and Table II) despite constant PTH infusion and virtually identical serum phosphorus levels in the four PTH infused groups (lines 2–5 of Table II). Log serum $1,25(OH)_2D_3$ was correlated inversely with Ca^{++} in the four groups infused with PTH ($r = -0.737$, $n = 31$, $P < 0.001$; $\log 1,25(OH)_2D_3 = -4.24 \times Ca^{++} + 6.95$) and also when the saline group is included ($r = -0.677$, $n = 41$, $P < 0.001$; $\log 1,25(OH)_2D_3 = -3.93 \times Ca^{++} + 6.52$).

Serum and blood gas measurements. Infusion of PTH alone increased total serum calcium and decreased serum phosphorus levels (Table II). Infusions of EGTA with PTH did not alter serum calcium or phosphorus levels compared with saline or PTH alone (Table II). PTH with 1.00 or 1.33 EGTA increased the serum magnesium level compared with saline. All three groups of rats infused with EGTA + PTH were alkalemic and hypocapnic compared with rats infused with saline (Table III).

Urine measurements. PTH infusion with or without EGTA increased urine calcium excretion (Table IV). All three infusions of EGTA with PTH increased urine phosphorus and depressed urine magnesium excretions (Table IV). The final weight of rats in the five groups did not differ (Table IV).

Discussion

Arterial blood ionized calcium concentration (Ca^{++}) clearly can exert a strong and independent control over serum $1,25(OH)_2D_3$ levels in the rat. A PTH infusion sufficient to raise Ca^{++} above normal depresses serum $1,25(OH)_2D_3$ even though PTH is present in excess of normal, and blood phosphorus level is reduced below normal. Lowering blood Ca^{++} through and below the normal range progressively raises serum $1,25(OH)_2D_3$ despite a constant, high PTH infusion and stable serum phosphorus levels. It is difficult to explain the suppression of serum $1,25(OH)_2D_3$ by PTH alone, and its progressive elevation by EGTA, except by arguing that Ca^{++} can down-regulate $1,25(OH)_2D_3$ production, or, possibly, increase $1,25(OH)_2D_3$ metabolic clearance rate.

There exist no in vivo studies in rats that are exactly comparable to ours. Low calcium diet raises serum $1,25(OH)_2D_3$, and could reduce Ca^{++} transiently; but low calcium diet also raises serum PTH level (5, 7, 10–12), so direct effects of Ca^{++} itself cannot be discerned. Thyroparathyroidectomized (TPTX) rats also may respond to low calcium diet with an increase in either serum $1,25(OH)_2D_3$ (11, 13) or tissue $1,25(OH)_2D_3$ localization (14). A response of serum $1,25(OH)_2D_3$ to low calcium diet despite TPTX supports our present result. Serum phosphorus level increases after TPTX, and rises during low calcium diet (11, 13), so the response of $1,25(OH)_2D_3$ to low calcium diet occurs despite hyperphosphatemia. Unfortunately, the ef-

Table II. Selected Serum and Blood Measurements

Group	n	$1,25(OH)_2D_3$	Ca^{++}	S Ca	S P	S Mg
		pg/ml	*mM*	*mg/dl*	*mg/dl*	*mg/dl*
Saline	10	35±6	1.26±0.02	9.9±0.1	8.1±0.4	1.6±0.1
PTH	11	12±3*	1.37±0.01*	10.5±0.1*	7.1±0.1*	1.9±0.1
PTH + 0.67 EGTA	7	90±33‡	1.20±0.01*‡	10.1±0.3	7.1±0.5	1.7±0.1
PTH + 1.0 EGTA	6	148±29*‡	1.19±0.03‡	10.4±0.4	7.0±0.6	2.1±0.1*
PTH + 1.33 EGTA	7	267±46*‡§‖	1.14±0.04*‡	10.4±0.3	7.0±0.8	2.4±0.2*‡§

Abbreviations: n, number of rats in each group; $1,25(OH)_2D_3$, serum 1,25 dihydroxyvitamin D_3; Ca^{++}, arterial blood ionized calcium; S, serum; Ca, calcium; P, phosphorus; Mg, magnesium. Saline, rats infused with 75 mM NaCl at 0.4 ml/h for 24 h; PTH, rats infused with 10 U/kg body wt/h of synthetic bovine 1–34 parathyroid hormone for 24 h; PTH + 0.67 EGTA, rats infused with PTH and 0.67 μm/min of EGTA for 24 h; PTH + 1.0 EGTA, rats infused with PTH and 1.0 μm/min of EGTA for 24 h; EGTA + 1.33 EGTA, rats infused with PTH and 1.33 μm/min of EGTA for 24 h; *, different than saline, $P < 0.05$; ‡, different than PTH, $P < 0.05$; §, different than PTH + 0.67 EGTA, $P < 0.05$; ‖, different than PTH + 1.0 EGTA, $P < 0.05$.

Table III. Arterial Blood Gas Measurements

Group	pH	H	P_{CO_2}	HCO_3
		neq/liter	*mmHg*	*meq/liter*
Saline	7.419±0.006	38±1	40±1	26±1
PTH	7.446±0.013	36±1	34±2*	23±1*
PTH + 0.67 EGTA	7.459±0.015*	35±1*	35±1*	25±1
PTH + 1.0 EGTA	7.467±0.017*	34±1*	34±2*	25±1
PTH + 1.33 EGTA	7.465±0.015*	34±1*	32±2*	23±1

Abbreviations: H, proton concentration; P_{CO_2}, partial pressure of carbon dioxide; HCO_3, bicarbonate concentration.
*, different than saline, $P < 0.05$; groups as in Table II.

fects of low calcium diet on Ca^{++} have not been measured longitudinally throughout an experiment to detect a transient reduction; by 12 d we (12) found normal values of Ca^{++}. Ca^{++} has not been specifically varied during low calcium diet. Therefore, the inference that Ca^{++} itself is the specific mediator of $1,25(OH)_2D_3$ increase during low calcium diet has not been directly tested. Serum $1,25(OH)_2D_3$ response to low calcium diet in TPTX animals is much below that of normal animals (11), perhaps because of the high serum phosphorus level, a low level of serum PTH, or some other factor. PTH raises both Ca^{++} and the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ in TPTX rats fed a vitamin D-deficient diet (32); this result is opposite to ours, in that Ca^{++} and $1,25(OH)_2D_3$ changed in parallel, not in opposition. However, TPTX rats are hypocalcemic to begin with, and do not become hypercalcemic with PTH as ours did.

An in vivo study by Hove et al. (33) supports our current findings. When PTH was infused into two TPTX lactating goats their serum calcium and $1,25(OH)_2D_3$ levels rose. However, infusion of calcium with the PTH increased serum calcium markedly and serum $1,25(OH)_2D_3$ fell. Although Ca^{++} was not measured in the experiment of Hove et al. (33) it probably increased during the calcium infusion.

Another experiment that supports the present results used chronic metabolic acidosis to alter Ca^{++} in intact rats eating a low calcium diet (12). Metabolic acidosis raised Ca^{++}, and prevented serum $1,25(OH)_2D_3$ from rising normally during the low calcium diet (12). When Ca^{++} was lowered with EGTA infusion, serum $1,25(OH)_2D_3$ rose to the levels normally achieved during a low calcium diet (21). There was a strong inverse correlation between serum $1,25(OH)_2D_3$ and Ca^{++} (12, 21), suggesting a suppressive effect of Ca^{++} itself on serum $1,25(OH)_2D_3$. The difficulty with these studies (12, 21) is that PTH was not precisely controlled. Even though PTH was not suppressed by high Ca^{++} during low calcium diet (12), judged by radioimmunoassay, minor changes in PTH that could have influenced $1,25(OH)_2D_3$ were not excluded.

In vitro, medium Ca^{++} seems a direct regulator of $1,25(OH)_2D_3$ production. Isolated chick tubules produce more $1,25(OH)_2D_3$ when medium calcium is lowered moderately, provided medium phosphorus is at least 1.2 mM (18); very low medium calcium levels, however, reduce $1,25(OH)_2D_3$ production (18). Chick mitochondria behave oppositely: raising medium calcium increases $1,25(OH)_2D_3$ production, but over a range of from 10^{-6} to 10^{-5} M, far below the regulatory range used for intact cells and only when pH is between 6.5 and 7.0 (20). These differences underscore the difficulties of comparing mitochondrial and cellular studies.

Superficially, at least, studies in humans appear to contradict our present findings: PTH increases serum $1,25(OH)_2D_3$ in normal people and in patients with hypoparathyroidism despite a rise in serum calcium (1, 4, 34, 35). Slovik et al. (34) found increased serum $1,25(OH)_2D_3$ 4 h after injection of synthetic 1–34 human PTH into normal people; Ca^{++} was not increased. At 12 and 24 h, $1,25(OH)_2D_3$ was elevated despite increased Ca^{++}; serum phosphorus level was reduced. This study is precisely comparable to ours, except for species, but with a different outcome: in the rat, the combination of increased serum PTH and Ca^{++}, and reduced serum phosphorus, suppressed serum $1,25(OH)_2D_3$; in humans, the same combination increased serum $1,25(OH)_2D_3$. The percentage declines in serum phosphorus in humans (~4.4–3.8 mg/dl, 14%) and our rats (8.1–7.1 mg/dl, 12%) are comparable, as are the increases in Ca^{++} (from ~1.27–1.37 mM, humans; 1.26±0.02 to 1.37±0.01 mM, rats) (34). Lambert et al. (1) found an increase of $1,25(OH)_2D_3$ in two normal people given parathyroid extract (PTE) despite increased serum calcium; but serum phosphorus level fell more—from 4 to 3 mg/dl in both—than in our study or in the study of Slovik et al. (34). Riggs et al. (35) found increased serum $1,25(OH)_2D_3$ in response to PTE but did not describe the changes in serum calcium or phosphorus levels, so their results cannot be compared with ours. Eisman et al. (4) described increased $1,25(OH)_2D_3$ and unchanged serum calcium and phosphorus levels 24 h after injection of PTE. Overall, the data suggest that there may be a species difference between rats and humans in relative sensitivity of $1,25(OH)_2D_3$ production to Ca^{++} vs. phosphorus level; compared with humans, rats seem to respond more to Ca^{++} than to serum phosphorus level.

Table IV. Selected Urine Measurements and Final Weights

Group	U cAMP/Cr	U Ca	U P	U Mg	Final wt
	nmol/mg	*mg/24 h*	*mg/24 h*	*mg/24 h*	*g*
Saline	18±2	2.4±0.3	24±2	9±1	259±4
PTH	21±3	5.2±0.5*	28±2	8±1	257±6
PTH + 0.67 EGTA	23±3	27±2*‡	33±2*‡	5±2*	254±6
PTH + 1.0 EGTA	21±3	45±6*‡§	43±5*‡§	3±1*‡	255±4
PTH + 1.33 EGTA	32±3*‡‖	49±3*‡§	45±2*‡§	4±2*‡	254±6

Abbreviations: U, urine; cAMP/Cr, cyclic AMP/creatinine; Ca, calcium; P, phosphorus; Mg, magnesium; wt, weight. *, different than saline, $P < 0.05$; ‡, different than PTH, $P < 0.05$; §, different than PTH + 0.67 EGTA, $P < 0.05$; ‖, different than PTH + 1.0 EGTA, $P < 0.05$. Groups as in Table II.

Primary hyperparathyroidism causes chronic high serum PTH levels and increased Ca^{++}, and serum levels of $1,25(OH)_2D_3$ commonly are high (36, 37). In general, the situation is comparable to the experiments of giving PTH to humans (1, 4, 34, 35) because serum phosphorus level is low. Oral phosphorus supplements can lower $1,25(OH)_2D_3$ without reducing PTH (38), supporting the notion that phosphorus depletion elevates $1,25(OH)_2D_3$ in hyperparathyroidism.

Overall, in the intact rat provided with a constant excess of PTH, blood ionized calcium level is a direct regulator of serum $1,25(OH)_2D_3$ and, perhaps, renal $1,25(OH)_2D_3$ production. Whether Ca^{++} has the same effects in humans, how ionized calcium actually affects serum $1,25(OH)_2D_3$, and whether Ca^{++} has a strong regulatory effect when PTH is normal, rather than high, remain unanswered questions. Since transepithelial active transport of calcium by the intestine is regulated by $1,25(OH)_2D_3$ (39, 40), an increase in the hormone augments calcium absorption and can thereby elevate the serum calcium level (41). Therefore, by directly regulating serum $1,25(OH)_2D_3$, calcium appears to control its own absorption.

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