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Original Paper

Hyperphosphatemia Modestly Retards Parathyroid Hormone Suppression during Calcitriol-Induced Hypercalcemia in Normal and Azotemic Rats

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Abstract
Background/Aims: In in vitro studies, a high phosphate concentration has been shown to directly stimulate parathyroid hormone (PTH) secretion in a normal calcium concentration and to reduce PTH suppression in a high calcium concentration. In hemodialysis patients during dialysis-induced hypercalcemia, the effect of hyperphosphatemia on PTH secretion was less than in vitro studies. Our goal was to determine whether hyperphosphatemia retards PTH suppression during calcitriol-induced hypercalcemia in azotemic rats with hyperparathyroidism. *Methods:* Rats underwent a two-stage 5/6 nephrectomy or sham operations. After surgery, rats received a high phosphate diet (P 1.2%, Ca 0.6%) for 4 weeks to induce hyperparathyroidism and then were placed on a normal diet (P 0.6%, Ca 0.6%) for two additional weeks to normalize serum calcium values in azotemic rats. At week 7, rats were divided into five groups and before sacrifice received at 24-hour intervals, three doses of calcitriol (CTR) or its vehicle. The five groups and dietary phosphate content were: group 1 – normal renal function (NRF) + 0.6% P + vehicle; group 2 – NRF + 0.6% P + CTR; group 3 – renal failure (RF) + 0.6% P + vehicle; group 4 – RF + 1.2% P + CTR; and group 5 – RF + 0.6% P + CTR. *Results:* In the two CTR-treated groups with marked hypercalcemia (groups 2 and 5), 15.52 ± 0.26 and 15.12 ± 0.13 mg/dl, respectively, stepwise regression showed that hyperphosphatemia retarded PTH suppression. When the two azotemic groups treated with CTR (groups 4 and 5) were combined to expand the range of serum calcium values, stepwise regression showed that hypercalcemia suppressed and hyperphosphatemia modestly retarded PTH suppression. Similarly, in groups 4 and 5 combined, correlations were present between PTH and both serum calcium (r = –0.70, p < 0.001) and serum phosphate (r = 0.64, p = 0.001). *Conclusions:* Hypercalcemia and high doses of calcitriol markedly reduced PTH secretion in azotemic rats despite severe hyperphosphatemia. Even though hyperphosphatemia did retard PTH suppression during hypercalcemia, its effect was small.

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Many previous studies have shown that phosphate loading or hyperphosphatemia induces skeletal resistance to parathyroid hormone (PTH) and, as a result, PTH secretion is stimulated [1–4]. Recent in vitro and in vivo studies have shown that a high phosphate concentration directly stimulates PTH secretion and prevents appropriate PTH suppression in a high calcium concentration [5–9]. In normal dogs in which the serum calcium concentration was clamped at a normal value, a phosphate infusion increased PTH secretion, but PTH secretion was not stimulated until the serum phosphate concentration had increased by approximately threefold [8]. Even then the increase in PTH secretion was transient and the effect of phosphate on PTH secretion was much less than that produced by hypocalcemia. In hemodialysis patients with hyperparathyroidism, an increased serum phosphate concentration was shown to prevent appropriate suppression of PTH secretion during dialysis-induced hypercalcemia, but the effect while significant was modest [9]. Thus, while recent evidence has shown that phosphate may directly stimulate PTH secretion, questions remain about the extent of its direct effect in vivo.

Calcitriol treatment is used in dialysis patients to lower PTH levels [10]. Besides its hypercalcemic effect, calcitriol also decreases PTH mRNA transcription [11]. Failure to control hyperphosphatemia in dialysis patients is associated with a poor response to calcitriol treatment [12], but it is unclear whether the poor response results from a direct stimulatory effect of phosphate on PTH secretion [5–8] or increased skeletal resistance to PTH [1–4]. In the former, phosphate may act at a post-transcriptional site to increase the stability of the PTH mRNA transcript [13]. In the latter, a decreased calcemic response to PTH results in the need for more PTH to maintain the same serum calcium value [4], an effect which presumably would increase PTH mRNA transcription. Our goal was to determine whether hyperphosphatemia affected the capacity of calcitriol-induced hypercalcemia to suppress PTH secretion in sham-operated and 5/6 nephrectomized rats with hyperparathyroidism.

Methods

Male Sprague-Dawley rats weighing 140–160 g underwent a two-stage 5/6 nephrectomy or sham operation [3, 4]. During surgery, rats were anesthetized with intraperitoneally administered ketamine 7.5 mg/100 g and xylazine 0.5 mg/100 g. Rats were housed in individual cages, given 15 g of food daily and allowed free access to water. Rats ingesting less than 12 g of food daily were removed from the study.

To stimulate PTH, all rats were initially given a high phosphate diet (P 1.2%, Ca 0.6%) [3, 4]. At week 5, the diet was changed to normal phosphate (P 0.6%, Ca 0.6%) for 2 weeks to normalize serum calcium values in azotemic rats. At week 7, rats were divided into five groups and either received intraperitoneal calcitriol (CTR, 500 pmol/100 g of body weight) or its vehicle at 72, 48 and 24 h before sacrifice. The first CTR dose was given 24 h after the rats were divided into groups. A high CTR dose was used to induce hypercalcemia because the study was designed to determine whether CTR together with hypercalcemia induced apoptosis of parathyroid cells [14]. The five study groups and dietary phosphate content given during Week 7 were: (1) group 1 – sham-operated with normal renal function (NRF) + 0.6% P diet + vehicle; (2) group 2 – NRF + 0.6% P diet + CTR; (3) group 3 – renal failure (RF) + 0.6% P diet + vehicle; (4) group 4 – RF + 1.2% P diet + CTR – only group given a high phosphate diet, and (5) group 5 – RF + 0.6% P diet + CTR. Both the 0.6 and 1.2% phosphate diets contained 0.6% calcium. Twenty-four hours after the last dose of CTR or vehicle, rats were sacrificed.

Serum calcium was measured with an autoanalyzer, serum phosphate with a specific kit (Sigma, St. Louis, Mo., USA), serum creatinine with a creatinine analyzer (Beckman, Fullerton, Calif., USA), and PTH with an immunoradiometric assay for the rat (Nichols, San Clemente, Calif., USA) [15]. Because of an insufficient quantity of serum, PTH values were not measured in one rat in groups 2 and 5.

Statistics
One-way ANOVA was used to compare multiple groups and the Fisher LSD was used for post-hoc analysis. The unpaired Student's t test was used to compare the two groups. For the correlation between two variables, the Pearson's correlation was used. $p < 0.05$ was considered significant. Stepwise regression was used to determine the effect of independent variables on a dependent variable. In this model, $p < 0.15$ was considered significant. In group 4, one rat was excluded from analysis because its serum phosphate (26.6 mg/dl), calcium (7.8 mg/dl), and PTH (97 pg/ml) values and weight at sacrifice (230 g) were more than two standard deviations from the group mean. Results are shown as mean ± SE.

Results

As shown in table 1, serum creatinine was greater in the 5/6 nephrectomized groups and was greatest in groups 4 and 5. Serum calcium was greater in the CTR-treated groups. Serum phosphate was greater in groups 4 and 5, but was also greater in group 4 (high phosphate diet) than in group 5. Parathyroid hormone was greater in groups 1 and 3 than in the hypercalcemic groups. In group 4, the weight at sacrifice was less than in groups 1, 2 and 3.

The effect of phosphate on PTH secretion was evaluated in several ways. In the two CTR-treated groups with similar degrees of hypercalcemia (table 1; group 2 vs. group 5, p = 0.34), PTH values were similar (p = 0.52) despite greater serum phosphate values in group 5 (p < 0.05). But while such results show that marked hypercalcemia induced by CTR treatment results in a profound

Table 1. Biochemical data and weights

	Group 1 NRF	Group 2 NRF + CTR	Group 3 RF	Group 4 RF + CTR	Group 5 RF + CTR	ANOVA p value
Diet	0.6% P	0.6% P	0.6% P	1.2% P	0.6% P	
n	12	13	12	12	12	
Serum						
Calcium, mg/dl	10.5 ± 0.1^a	15.5 ± 0.2^b	10.7 ± 0.1^a	13.4 ± 0.1^c	15.1 ± 0.3^b	<0.001
Phosphate, mg/dl	6.8 ± 0.2^a	8.6 ± 0.6^a	7.5 ± 0.4^a	13.4 ± 1^b	1.4 ± 1.2^c	<0.001
PTH, pg/ml	72 ± 7^a	8 ± 1^b	178 ± 42^c	12 ± 2^b	7 ± 1^b	<0.001
Creatinine, mg/dl	0.40 ± 0.04^a	0.51 ± 0.05^a	0.66 ± 0.07^b	1.04 ± 0.08^c	1.18 ± 0.14^c	<0.001
Weight, g	302 ± 8^a	301 ± 4^a	301 ± 8^a	278 ± 5^b	289 ± 6^a	= 0.03

Mean values ± SE.
NRF = Normal renal function; CTR = calcitriol; RF = renal failure; P = phosphate.
Differences between individual groups determined by post-hoc test.
[a] Value not different from other groups marked with [a]. [b] Value not different from other groups marked with [b]; $p < 0.05$ vs. [a] except for weight in which there was no difference between groups 4 and 5. [c] Value not different from other groups marked with [c]; $p < 0.05$ vs. [a] and [b].

reduction of PTH values, it does not eliminate the possibility that hyperphosphatemia is exerting an effect. To evaluate a potential effect of hyperphosphatemia on PTH secretion during CTR-induced hypercalcemia, stepwise regression with PTH as the dependent variable and serum calcium and phosphate as the independent variables was performed in each of the three CTR-treated groups (groups 2, 4, and 5) and in the two CTR-treated, azotemic groups combined (groups 4 and 5). The latter was performed to expand the range of serum calcium values.

In groups 2 and 5, each with marked hypercalcemia, stepwise regression showed that serum phosphate retarded PTH suppression but serum calcium was without effect (table 2A, B). In group 4, with a lesser degree of hypercalcemia than groups 2 and 5, an increase in serum calcium suppressed PTH secretion and an increase in serum phosphate retarded PTH suppression (table 2C). When the two hypercalcemic azotemic groups (groups 4 and 5) were combined, an increase in serum phosphate retarded PTH suppression and an increase in serum calcium decreased PTH secretion (table 2D). In these two groups, correlations between PTH and both serum calcium (r = –0.70, p < 0.001) and phosphate (r = 0.64, p = 0.001) are shown in figure 1.

Table 2. Stepwise regression in calcitriol-treated rats

|	t value	p value
A Group 2 (calcitriol treated with normal renal function)		
Dependent variable – serum PTH		
Independent variables		
Serum calcium	0.1	NS
Serum phosphate	2.9	0.015
	$r^2 = 0.46$	
B Group 5 (calcitriol treated with renal failure)		
Dependent variable – serum PTH		
Independent variables		
Serum calcium	0.6	NS
Serum phosphate	2.6	0.03
	$r^2 = 0.42$	
C Group 4 (calcitriol treated with renal failure and on high phosphate diet)		
Dependent variable – serum PTH		
Independent variables		
Serum calcium	–2.1	0.07
Serum phosphate	2.3	0.04
	$r^2 = 0.69$	
D Groups 4 and 5 combined		
Dependent variable – serum PTH		
Independent variables		
Serum calcium	–2.9	0.009
Serum phosphate	2.1	0.04
	$r^2 = 0.58$	
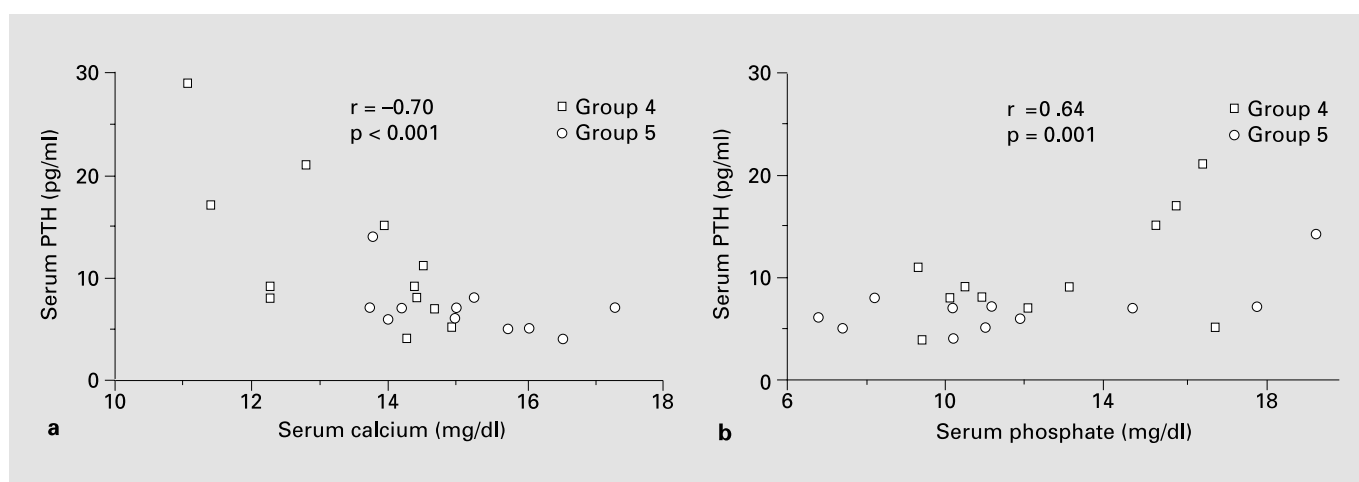
Fig. 1. Correlation between PTH and both serum calcium and phosphate in azotemic rats given calcitriol. **a** An inverse correlation is present between PTH and serum calcium in azotemic rats given calcitriol (groups 4 and 5). **b** A direct correlation is present between PTH and serum phosphate in azotemic rats given calcitriol (groups 4 and 5).

Discussion

Because all rats received the same diets during the first 6 weeks, the biochemical values at the onset of week 7 should be similar in the two nonazotemic (groups 1 and 2) and the three azotemic groups (groups 3, 4, and 5). Moreover, biochemical values at sacrifice in groups 1 (nonazotemic) and 3 (azotemic) should reflect the biochemical values present in nonazotemic (group 2) and azotemic (groups 4 and 5) groups before CTR was given 72 h earlier. It is also possible that CTR administration and hypercalcemia may have contributed to the increased serum creatinine values at sacrifice in groups 4 and 5. The former acts by inhibiting renal creatinine secretion [16] and the latter by inducing volume depletion.

In our study, serum calcium had to be increased by more than 3 mg/dl before maximal PTH suppression was observed. In previous studies in normal rats [17, 18], rabbits [19], cows [20], dogs [21], and humans [22], the increase in serum calcium needed to achieve maximal PTH suppression was less than 1 mg/dl. Even in primary hyperparathyroidism [23] or secondary hyperparathyroidism due to vitamin D deficiency [24] or renal failure [25, 26], the increase in serum calcium needed to maximally suppress PTH secretion was approximately 1 mg/dl. But in azotemic rats on a high phosphate diet and with marked hyperphosphatemia [26], the increase in serum calcium needed for maximal PTH suppression was similar to that in our study. Thus, even though hypercalcemia

and high CTR doses markedly reduced PTH values in our study, the magnitude of hypercalcemia necessary to achieve maximal PTH suppression was greater than expected and as suggested by stepwise regression, was probably due to the hyperphosphatemia.

Our results showing that hyperphosphatemia retards PTH suppression, but a marked elevation in the serum calcium concentration still maximally suppresses PTH secretion are consistent with recent in vitro studies which have also suggested a potential mechanism. In the parathyroid gland, a high extracellular calcium concentration is coupled to phospholipase A_2 activation and formation of arachidonic acid, a potent inhibitor of PTH release. A high phosphate concentration decreased arachidonic acid production and prevented PTH suppression by a high (1.35 mM) calcium concentration [27]. But adding a calcium ionophore to this same solution increased arachidonic acid levels and suppressed PTH secretion [28]. These results suggest that a high phosphate concentration interferes with the increase in the intracellular calcium concentration achieved with the 1.35 mM calcium concentration, but further increasing intracellular calcium can overcome its inhibitory effect. Finally, in azotemic rats, treatment with a calcimimetic maximally suppressed PTH levels despite a doubling of serum phosphate values [29] suggesting that a hypercalcemic equivalent can suppress PTH secretion despite hyperphosphatemia.

Besides a direct effect of phosphate, another potential factor in the delayed PTH suppression during hypercal-

cemia could be changes in the calcium receptor. A reduction in the calcium receptor in the parathyroid gland has been shown to be present in 5/6 nephrectomized rats on a high phosphate diet [30]. But a reduction in the calcium receptor is also present in patients with primary and secondary hyperparathyroidism [31, 32] and still these patients achieve maximal PTH suppression with relatively small increases in the serum calcium concentration [23–26].

The primary effect of CTR is on PTH mRNA transcription [11, 33] while that of calcium and phosphate is post-transcriptional [13]. Although we used a high CTR dose, maximal suppression of PTH mRNA transcription has been achieved in normal and azotemic rats with a CTR dose of 25 pmol/100 g [34, 35]. Thus, our higher CTR dose should not have had a greater effect. We also do not believe that the CTR dose used so drastically reduced PTH mRNA transcription that subsequent transcripts were unavailable to be acted on by phosphate. In azotemic rats given CTR, serum PTH values progressively decreased as the magnitude of hypercalcemia increased. If high CTR doses had totally extinguished PTH mRNA transcription, the graded effect on PTH secretion shown for hypercalcemia would not have been expected. Secondly, hyperphosphatemia retarded PTH suppression in all CTR-treated groups. If CTR had totally blocked PTH mRNA transcription, it would seem that hyperphosphatemia, which acts post-transcriptionally, would not have an effect. But because CTR does inhibit PTH mRNA transcription, the effect of hyperphosphatemia on PTH secretion in non-CTR induced hypercalcemia might be even greater.

The normal calcium-phosphate product in the rat is approximately 80, a value twice that in humans. In groups 4 and 5, the calcium-phosphate product represents a doubling of the normal product, a situation often encountered in dialysis patients. To know whether hypercalcemia suppresses PTH normally during hyperphosphatemia, both hypercalcemia and hyperphosphatemia must be present. A high CTR dose and hypercalcemia should maximize PTH suppression and thus help to determine whether hyperphosphatemia has an independent effect. Another potential concern is whether the high calcium-phosphate product could affect the biological activity of the measured calcium. Even though the serum phosphate concentration and the calcium-phosphate product were greater in group 5 than in group 2, a similar magnitude of hypercalcemia produced a similar suppression of PTH. This suggests that the serum calcium concentration in the two groups had a similar degree of biological activity. Further support for the equivalence of biological activity is from CTR-treated azotemic rats in which PTH levels inversely correlated with the degree of hypercalcemia. Thus, despite hyperphosphatemia our results show that PTH secretion responded to the measured serum calcium concentration.

In conclusion, hypercalcemia and high doses of calcitriol markedly reduced PTH secretion in azotemic rats with hyperparathyroidism despite severe hyperphosphatemia. Even though our results show that hyperphosphatemia exerted an independent effect, its effect was small.

Acknowledgments

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