Acute regulation of parathyroid hormone by dietary phosphate

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Martin, Daniel R., Cynthia S. Ritter, Eduardo Slatopolsky, and Alex J. Brown. Acute regulation of parathyroid hormone by dietary phosphate. Am J Physiol Endocrinol Metab 289: E729–E734, 2005.—Secondary hyperparathyroidism in chronic renal failure is stimulated by dietary phosphate (Pi) loading and ameliorated by dietary Pi restriction. We investigated the rapidity of the response of serum parathyroid hormone (PTH) to changes in dietary Pi. When uremic rats adapted to a high Pi diet (HPD) were fed a single meal of low Pi, diet (LPD), plasma PTH fell 80% within 2 h; plasma Pi fell 1 mg/dl with no change in plasma ionized Ca (ICa). When uremic rats on the HPD were gavaged with LPD, PTH fell 60% within 15 min; plasma Pi fell by 3.0 mg/dl with no change in total plasma Ca. However, HPD gavage increased PTH by 80% within 15 min with no change in plasma Pi or Ca, suggesting that the response may be independent of altered plasma Pi. Duodenal infusion of sodium Pi increased PTH twofold within 10 min, with no change in ICa but an increase in plasma Pi; whereas duodenal infusion of NaCl had no effect on any of these parameters. Intravenous infusion of sodium phosphate also increased PTH within 10 min with no change in plasma ICa; intravenous NaCl had no effect. Additionally, duodenal infusion of phosphonoformate, a nonabsorbable phosphate analog, increased PTH fourfold within 5 min, but did not change plasma P or ICa. These findings indicate that oral Pi increases PTH release in vivo more rapidly than previously reported; this response may be from both plasma phosphate and an additional signal arising from the gastrointestinal tract.

phosphate; gastrointestinal tract

SECONDARY HYPERPARATHYROIDISM (2°HPT) is a common disorder in patients with chronic renal failure (26, 30). The increased parathyroid hormone (PTH) secretion and parathyroid gland hyperplasia of 2°HPT are attributed primarily to the retention of phosphate resulting from the loss of renal function (9, 15, 18, 29, 34). The hyperphosphatemia and loss of functional renal mass lead to decreased production of 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], an inhibitor of PTH gene transcription (24, 27, 28) and parathyroid cell growth (8, 13, 32). The hyperphosphatemia reduces the levels of serum Ca, and low 1,25(OH)₂D₃ reduces the rate of intestinal Ca absorption; these two factors produce a tendency toward hypocalcemia, which is a strong stimulus for 2°HPT. The primary role of dietary phosphate is evident from the demonstration that phosphate restriction can prevent the development of 2°HPT in rats after subtotal nephrectomy (9) and can arrest 2°HPT in uremic rats with established 2°HPT (33). Furthermore, by careful balance of the dietary Ca and phosphate levels, it has been shown that dietary phosphate can regulate parathyroid gland function independently of changes in serum Ca or 1,25(OH)₂D₃ (15).

The mechanism by which dietary phosphate controls 2°HPT is not clear. One hypothesis is that phosphate may regulate the expression of the Ca-sensing receptor (CaR) and influence Ca sensing by the parathyroid glands. This was based on the observations by several laboratories that CaR expression is reduced in the parathyroid glands of patients with chronic renal failure (7, 10–12, 35). We found that a high dietary phosphate diet that promotes 2°HPT led to downregulation of the CaR (3) and reduced the sensitivity of the parathyroid glands to suppression of PTH levels by Ca (i.e., elevates the Ca set point; see Ref. 23). Conversely, a low-phosphate diet that prevents and arrests 2°HPT maintained (22) and restored (23) normal expression of the CaR and normalized the Ca set point (23). However, time course experiments revealed that the reduction in CaR content with high-phosphate diet occurred after the initiation of 2°HPT and that restoration of CaR expression with a low-phosphate diet occurred well after the arrest of 2°HPT (23). These findings suggested that altered CaR expression is likely secondary to changes in parathyroid cell growth. This would be consistent with the downregulated CaR in primary hyperparathyroidism, in which parathyroid glands grow autonomously due to genetic abnormalities or mutations.

The most striking observation in the time course studies (23) was the very rapid response of plasma PTH levels to changes in dietary phosphate. We observed that changing the diet of uremic rats from high phosphate to low phosphate normalized PTH levels within 1 day. The rapidity of the response had not been reported previously. In the present study, we have further investigated this acute response of PTH to dietary phosphate. Our findings indicate that oral phosphate can regulate plasma PTH within minutes, faster than the direct effect of phosphate observed in parathyroid cultures. Furthermore, we provide evidence that part of this effect may be attributed to a novel signal arising from the gastrointestinal tract.

METHODS

Animals. All studies were performed on female Sprague-Dawley rats (250–300 g) obtained from Harlan (Indianapolis, IN). Initial studies employed uremic rats that had undergone a one-stage subtotal (5⁄6) nephrectomy, as described previously (3). All animal protocols were approved by the Animal Studies Committee of Washington University School of Medicine.

Timed feeding experiment. Uremic rats were maintained for 2 wk on a high-phosphate diet (HPD) containing 0.9% P and 0.6% Ca (ad libitum). The rats were then placed on a timed-feeding schedule with the HPD available only between 9:00 A.M. and 11:00 A.M. for 2 wk. This period was necessary to allow adaptation to the restricted time of feeding. On the day of study, a blood sample was taken from the tail vein under light ether anesthesia immediately before feeding a low-phosphate diet (LPD) containing 0.2% P and 0.5% Ca. Additional...
ionized Ca, P, and PTH.

were collected at the specified times, and plasma was analyzed for duodenally infused with the designated substance. Blood samples were taken immediately after the feeding period (2 h) and at 5, 8, and 12 h after feeding and analyzed for total Ca, phosphorus, and PTH. Data are expressed as means ± SE; n = 6. *P < 0.05 vs. time 0.

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Gavage experiments. Uremic rats were fed the HPD for 1 mo (ad libitum). The rats were anesthetized under ether, and a blood sample was taken from the tail vein. The rats were then gavaged using a pediatric feeding tube with 2 ml of a 50% aqueous slurry of the HPD or the LPD or 0.25 ml of 1 M sodium phosphate, pH 7.0. Additional blood samples were taken at the specified times for total Ca, P, and PTH.

Duodenal phosphate infusion. Normal rats were anesthetized under ether, and a midline incision was performed, and a cannula was placed through the duodenal wall just distal to the pylorus. The right femoral artery was catheterized for blood collection. After recovery from anesthesia, baseline blood samples were taken, and the rats were duodenally infused with the designated substance. Blood samples were collected at the specified times, and plasma was analyzed for ionized Ca, P, and PTH.

Effect of duodenal phosphate infusion on PTH clearance. The clearance rate of PTH after duodenal infusion of phosphate or saline was examined. Rats were prepared as outlined above, except that an additional catheter was placed in the left femoral vein. After recovery from anesthesia, the rats were infused with 60 μmol sodium phosphate (pH 7.0) or sodium chloride (n = 5/group). Later (5 min), 100 ng human PTH-(1–84) was injected via the venous catheter. Blood samples were taken via the arterial catheter after 2, 5, 10, 20, and 40 min and analyzed for human PTH using the Nichols Intact PTH kit that does not recognize endogenous rat PTH.

Intravenous phosphate infusion. Normal rats were anesthetized under ether, and catheters were placed in the right femoral artery for blood collection and left femoral vein for the infusion. After recovery from the anesthesia, the rats were infused with 50 mM sodium phosphate or 50 mM sodium chloride for 30 min. Blood samples were taken at 0, 5, 10, 20, and 30 min of infusion. Plasma was analyzed for ionized Ca, P, and PTH.

Serum chemistries. Plasma samples were analyzed for total Ca using an atomic absorption spectrophotometer (model 1100B; PerkinElmer, Downers Grove, IL). Ionized Ca was measured with a specific electrode on a Nova 8 electrolyte analyzer (Waltham, MA). Plasma P was determined with Cobas Mira Plus (Roche Diagnostic Systems, Branchburg, NJ). Rat plasma PTH was measured using either a rat PTH immunoradiometric assay kit (Immutopics, San Clemente, CA) or a rat PTH ELISA (Scantibodies Laboratories, Santee, CA). The latter assay, which does not recognize rat PTH-(7–84), was used for the gavage and duodenal infusion experiments.

Statistics. Data are presented as means ± SE. Changes in plasma chemistries were analyzed by repeated-measures ANOVA using the Instat program (GraphPad Software, San Diego, CA).

RESULTS

Our previous studies revealed that the elevated PTH levels in uremic rats fed a HPD (0.9% P) could be normalized within 1 day of feeding an LPD (0.2% P; see Ref. 23). To determine the time required for the correction of PTH by LPD, timed feeding and gavage experiments were performed in uremic rats adapted to the HPD.

Timed feeding experiment. Uremic rats were fed the HPD ad libitum for 2 wk postnephrectomy and then placed on a timed feeding schedule for 2 wk that provided diet only for a 2-h period each morning (9:00 A.M. to 11:00 A.M.). On the day of the study, blood samples were taken from the tail immediately before the feeding period (time 0) and then again after 2 (end of feeding period), 5, 8, and 12 h. Figure 1 shows the plasma levels of PTH and the changes in phosphorus and Ca. By the end of the 2-h feeding period, PTH levels reached a nadir. This was accompanied by a similar rapid fall in plasma phosphorus. Total plasma Ca did not change significantly during the course of the experiment.

Gastric gavage. To further define the time required for LPD to reduce PTH, uremic rats adapted to the HPD were gavaged with 50% aqueous slurries of either LPD or HPD. Figure 2 shows that the PTH dropped to a minimum by 15 min after gavage with LPD. This was accompanied by a fall in plasma...
phosphorus (Fig. 2A), but no change in plasma Ca was observed (data not shown). PTH remained at this nadir throughout the 60-min time course. In contrast, gavage with the HPD increased PTH by 80% by 15 min with no change in plasma phosphorus (Fig. 2B) or plasma Ca (data not shown). PTH remained elevated at 30 min but fell to near basal levels by 60 min, and this was accompanied by a fall in plasma phosphorus.

**Duodenal infusion of sodium phosphate.** The preceding experiments indicated that the acute effects of high dietary phosphate on PTH levels may be independent of changes in plasma phosphate. It is known that nutrient sensing occurs in the gastrointestinal tract, typically in the upper small intestine. Therefore, we investigated the effect of introducing a solution of sodium phosphate vs. sodium chloride directly in the duodenum, rather than dietary slurries of HPD and LPD. In addition, these experiments utilized normal rats rather than uremic rats to determine if the acute response to phosphate is a normal physiological mechanism or is confined to the pathological condition of renal failure and/or hyperparathyroidism.

Infusion of sodium phosphate (60 μmol) directly in the duodenum of normal rats produced a prompt twofold increase in PTH between 5 and 10 min, and PTH remained elevated throughout the 30-min experiment (Fig. 3). Infusion of an equimolar amount of sodium chloride had no significant effect on PTH. Figure 3B shows the changes in plasma phosphorus and ionized Ca in rats infused duodennally with sodium phosphate or sodium chloride. Neither agent altered ionized Ca. Plasma phosphorus was gradually increased after sodium phosphate infusion, reaching a statistical significance at 30 min, but plasma phosphorus was not affected by sodium chloride infusion.

**Effect of duodenal phosphate infusion on PTH clearance.** The acute effects of phosphate on circulating PTH levels could potentially involve altered secretion, clearance, or both. To determine if PTH clearance was affected by duodenal phosphate, human PTH was injected intravenously 5 min after duodenal infusion of 60 μmol sodium phosphate (pH 7.0) or sodium chloride. Arterial blood samples were analyzed for human PTH using a kit that does not recognize rat PTH. As shown in Fig. 4, PTH clearance curves were not different in the two groups.

**Acute effect of duodenal infusion of phosphonoformate on PTH.** The preceding protocol demonstrated that duodenal infusion of a sodium phosphate solution can acutely increase plasma PTH in normal rats but that this was accompanied by an increase in serum phosphorus. In an attempt to block phosphate absorption, we examined the effects of phosphonoformate, a nonabsorbable phosphate analog that blocks cellular uptake of phosphate, on PTH. As shown in Fig. 5A, phosphonoformate alone produced a very prompt fourfold increase in PTH at 5 and 10 min postinfusion, but PTH declined thereafter. Because of the lower solubility of phosphonoformate, only 30 μmol could be dissolved in the 0.25-ml infusate. An equimolar infusion of sodium phosphate produced a slower increase in PTH that was maximal at 10 min. Figure 5B shows that this lower dose of phosphate also led to a gradual increase in plasma phosphorus over the 30-min protocol. Phosphonoformate had no effect on plasma phosphorus during the first 10 min when PTH was increased but produced a slight, but not significant, elevation at later times. Figure 5C shows that neither phosphate nor phosphonoformate altered ionized Ca.

**Intravenous phosphate infusion.** Because duodenal infusion produced an increase in plasma P that paralleled the elevation in PTH, we investigated the potential role of plasma phosphate on PTH by direct intravenous infusion. Normal rats infused with sodium phosphate had an increase in PTH within 10 min (Fig. 6A) that was accompanied by the expected elevation in plasma phosphate, but no change in ionized Ca (Fig. 6B). Sodium chloride had no effect on any of these parameters.

**DISCUSSION**

The mechanisms by which dietary phosphate regulates physiological processes are very poorly understood. Dietary phos-
phosphate can modulate the renal sodium-phosphate cotransporter (NPT2a; see Refs. 5 and 14) and is well-known to influence PTH levels in renal failure patients. Questions as to whether phosphate acts directly or via changes in serum Ca or 1,25(OH)2D3 have been raised over the years. Direct effects of phosphate have been documented on NPT2a activity in cultured proximal tubular (opossum kidney) cells (2, 4, 6, 20, 21) and, more recently, on PTH synthesis by rat parathyroid explants (1, 31) and bovine parathyroid gland slices (19). However, other mechanisms for the control of NPT2 and PTH by dietary phosphate cannot be excluded. The direct effects of phosphate on PTH release in parathyroid cultures required 10 min (1, 19, 31). In contrast, the stimulation of PTH by phosphate observed in the present in vivo study occurred within 10 min. This implies that the mechanism is distinct from the regulation of PTH release by phosphate in vitro. Furthermore, clearance measurements indicated that the rapid increase in plasma PTH was not the result of altered clearance, but rather an effect on PTH release by the parathyroid glands.

The acute regulation of PTH by dietary phosphate, by duodenally infused phosphate, and by intravenously infused phosphate did not produce any detectable alterations in plasma ionized Ca, even at the very early time points measured. This would appear to exclude Ca as a mediator of the effects of phosphate, although we cannot rule out the possibility that fluctuations in plasma Ca by phosphate were immediately corrected by PTH.

Introduction of phosphate directly in the duodenum produced simultaneous increases in plasma levels of PTH and phosphate. The role of the increase in plasma phosphate in the release of PTH was investigated by two approaches. The intravenous infusion of phosphate to achieve increments in plasma phosphate comparable to those produced by intraduodenal phosphate infusion indicated that changes in plasma phosphate can indeed alter PTH acutely. The second approach involved the inhibition of the absorption of intraduodenal phosphate using phosphonoformate, an inhibitor of sodium phosphate cotransporters. Surprisingly, phosphonoformate itself was found to acutely increase plasma PTH. This poorly absorbed phosphorus-containing compound did not alter plasma Ca or phosphate levels. Thus it appeared that phosphonoformate is capable of signaling the parathyroid glands directly through a signal emanating from the gastrointestinal tract. It is especially interesting that the increase in PTH after phosphonoformate infusion is transient. Pulsatile increases in circulating PTH after PTH administration have been shown to produce anabolic effects on bone. Thus these findings may have significance for the treatment of metabolic bone diseases, including osteoporosis.
Dietary phosphate also acutely controls renal phosphate reabsorption (5, 14). Increases in dietary phosphate decrease the renal type II sodium-dependent NPT2a within 2 h with no increase in serum phosphate (14), whereas low-phosphate diet increases NPT2a within 2 h, accompanied by a fall in serum phosphate (5, 14). It has been speculated that the acute effects of dietary phosphate are independent of serum phosphate and may be mediated by a novel signal (17). Although the effects of dietary phosphate on renal phosphate reabsorption are partly independent of PTH, the acute actions of dietary phosphate in the parathyroid glands alter PTH in the appropriate direction to aid the kidney in properly handling the incoming phosphate load from the diet.

The nature of the phosphate “sensor” has been a subject of great interest. The regulatory effects of dietary phosphate on a number of processes implicate the existence of such a sensor, but its locations and properties have remained elusive. Our findings indicate that the small intestine may contain a sensor that responds to phosphonoformate. This sensing mechanism may also respond to phosphate itself, but this is difficult to determine in vivo because of the rapid absorption of phosphate by the small intestine and the ability of plasma phosphate to stimulate PTH release. The parathyroid glands may sense the intravenous phosphate load directly or indirectly. As mentioned above, however, the response observed in vivo is more rapid than that seen in organ culture, suggesting a distinct sensing mechanism. The sensing may involve a cell surface receptor like that for Ca, or it could involve metabolic flux such as that for glucose sensing (16, 25). The latter possibility appears to be the case for phosphate sensing by vascular smooth muscle cells. Increased media phosphate stimulates expression of the osteoblastic genes (cbfα1 and osteocalcin) but the effect is blocked by phosphonoformate, which inhibits phosphate uptake by NPT2a. In similar studies performed in rat parathyroid organ culture, however, phosphonoformate did not inhibit the direct (3-h) effect of extracellular phosphate (our unpublished observation).

In summary, we have found that dietary phosphate can acutely control PTH release by the parathyroid glands within 10 min by a mechanism that is independent of changes in plasma Ca but may involve the concomitant changes in plasma phosphate. The direct intravenous infusion of phosphate elevated PTH, but the rapidity of the response is faster than that reported in parathyroid organ culture, suggesting a distinct mechanism. On the other hand, the ability of phosphonoformate to acutely increase PTH levels in the absence of changes in plasma Ca and phosphate provides evidence for a signal derived from the gastrointestinal tract that can trigger PTH release. The mechanism for sensing phosphonoformate, and perhaps phosphate, in the gastrointestinal tract and the downstream signaling is under investigation. This novel pathway for acute phosphate control of PTH release is not only of physiological and pathological importance but may lead to new pharmacological interventions for the treatment of hyperparathyroidism and bone disease.

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REFERENCES


