Dopamine Enhances the Phosphaturic Response to Parathyroid Hormone in Phosphate-Deprived Rats

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ABSTRACT
Phosphate deprivation results in a resistance to the phosphaturic effect of parathyroid hormone. Dopamine is phosphaturic and is synthesized by kidney proximal tubule, the nephron subsegment where parathyroid hormone inhibits phosphate transport. Thus, to test the hypothesis that phosphate deprivation is associated with low intrarenal dopamine synthesis and that dopamine infusion will overcome the resistance to the phosphaturic response to parathyroid hormone, the following study was performed. The effect of dietary phosphate intake on intrarenal dopamine synthesis, as reflected by urinary dopamine excretion, was determined. Rats were placed in metabolic cages (N = 5) and were fed a low-phosphate diet (0.07% P) for 4 days and then a high-phosphate diet (1.8% P) for 4 days. Twenty-four-hour urinary dopamine excretion was significantly lower in rats fed a low-phosphate diet (2.53 ± 0.06 versus 4.10 ± 0.30 µg/day). Further, the effect of dopamine infusion on the blunted phosphaturic response to parathyroid hormone was studied in rats fed a low-phosphate diet for 1, 2, and 3 days. Control clearances were taken 2 h after thyroparathyroidectomy; then, parathyroid hormone (33 U/kg plus 1 U/kg/min), dopamine (25 µg/kg/min), or parathyroid hormone plus dopamine were infused for 60 min. Changes in the fractional excretion of phosphate were significantly greater in rats fed a low-phosphate diet infused with parathyroid hormone plus dopamine than in rats fed a low-phosphate diet infused with parathyroid hormone alone (Δ27.9 ± 5.8 versus 11.2 ± 2.6% for day 1; 28.4 ± 1.4 versus 7.1 ± 3.6% for day 2; and 10.7 ± 2.8 versus -0.2 ± 0.2% for day 3; N = 5 for all groups). It was concluded that, during short-term phosphate deprivation, intrarenal dopamine synthesis is low and dopamine infusion enhances the phosphaturic response to parathyroid hormone.

Key Words: Dopamine, phosphate, parathyroid hormone

Parathyroid hormone (PTH) is phosphaturic and inhibits phosphate transport by the proximal tubule (1,2). Rats fed a low-phosphate diet (LPD) exhibit a well-established resistance to the phosphaturic effect of PTH by mechanisms not yet fully understood (3,4).

Extraneural dopamine (DA) is synthesized by kidney proximal tubular cells (5), the same nephron subsegment where PTH inhibits phosphate transport. Aromatic L-amino acid decarboxylase, the enzyme responsible for the conversion of L-dopa to DA, has been well characterized in the kidney of the rat, and its' activity has been demonstrated in the proximal convoluted tubule and pars recta subsegments of the rat nephron (6). DA infusion has been shown to increase phosphate excretion. This effect was reproduced by the infusion of L-dopa, and a role for intrarenal DA in the regulation of phosphate reabsorption was suggested (7).

We propose a role for intrarenally synthesized DA in the resistance to the phosphaturic effect of PTH during short-term phosphate deprivation. Therefore, this study was performed to test the hypothesis that intrarenal DA synthesis is low in rats fed a LPD and that DA infusion enhances the phosphaturic response to PTH administration in short-term phosphate-deprived rats.

METHODS
Effect of Changes in Dietary Phosphate Intake on Urinary DA Excretion
Metabolic balance studies were performed in conscious male Sprague-Dawley rats (N = 5) that were fed LPD (0.07% P; ICN Pharmaceutical) for 4 days and then changed to a high-phosphate diet (HPD; 1.8% P) for 4 days. The HPD was prepared by supplementing the standard LPD with a mixture of sodium and potassium phosphate (ratio of monobasic:dibasic salts of 1:4), to a final content of 1.8%
P. For rats fed the LPD, sodium and potassium were supplemented to the same concentration as in the HPD. Rats were monitored to assure a daily ingestion of 12 g of the respective diet. Urine samples were collected every 24 h in a container in dry ice with 5 mL of 33% acetic acid to prevent catecholamine degradation. For measurements of urinary-free DA, the samples were filtered and purified by a modification of the method of Sharpless et al. (8). Quantification of urinary DA was then determined by HPLC with electrochemical detection (9). Free urinary DA excretion is a well-established reliable index of intrarenal DA synthesis (10–12). Recoveries of added standards in all of the extractions averaged 80 to 95%. Urinary sodium concentrations were measured by an ion-selective electrode (Beckman E2A; Beckman Instruments, Fullerton, CA). Phosphate concentrations were determined by the method of Chen et al. (13).

Effect of DA Infusion on the Resistance to the Phosphaturic Effect of PTH

Male Sprague-Dawley rats weighing 200 to 300 g, from Harlan-Sprague-Dawley (Indianapolis, IN), fed 12 to 15 g/day of LPD for 1, 2, and 3 days (0.07% P; ICN Pharmaceutical) or normal phosphate diet (NPD; 0.7% P), with free access to water, were used in this study. The NPD was prepared by supplementing the standard LPD with sodium and potassium phosphate. The ratio of monobasic-to-dibasic salts was 1:4, giving a final concentration of 0.7% P. For animals fed LPD, sodium and potassium contents were supplemented by NaCl and KCl to the same concentration as in NPD. Rats were anesthetized by a 100-mg/kg body wt i.p. injection of 5-sec-butyl-ethyl-2-thyobarbituric acid (Inactin; BYK Golden, Konztanz, Germany) and were placed on a heated table to maintain body temperature between 36 and 38°C.

Rats were acutely thyroparathyroidectomized by heat cautery, and then a tracheostomy was performed. Polyethylene catheters (PE-50) were placed in the left and right jugular veins for infusions and in the left carotid artery for monitoring mean arterial pressure and collection of blood samples. A PE-90 catheter was placed in the bladder for collection of urine samples.

An i.v. infusion of a solution of 3% inulin in saline (0.9% NaCl) and of saline (0.9% NaCl), each at a rate of 1 mL/100 g body wt, were started. A 1-h equilibration period was allowed after the surgery. Control clearances of 30 min were begun 2 h after thyroparathyroidectomy. After the control clearance was taken, infusion of an appropriate experimental solution was started. Experimental clearances of 30 min were initiated an hour later. Animals infused with DA also received a volume replacement with saline solution (0.9% NaCl), at a rate of 1.5 mL/h.

Twelve groups of rats were studied, and the effects on phosphate excretion were determined.

RATS FED A NPD

(A) Effect of PTH (N = 5)

After the control clearance, a maximally phosphaturic dose of PTH was given with an initial bolus of 33 U/kg, followed by a continuous infusion at a rate of 1 U/kg/min.

(B) Effect of PTH in the presence of DA (N = 5)

PTH was given as described in protocol A. In addition, a simultaneous infusion of a pharmacological dose of DA (25 μg/kg/min) was given.

(C) Effect of DA (N = 5)

DA was infused as described in protocol B. The same experimental infusions and doses used in protocols A, B, and C, for rats fed a NPD, were followed for rats fed a LPD for 1, 2, and 3 days.

RATS FED LPD FOR 1 DAY

(A) Effect of PTH (N = 5)

(B) Effect of PTH in the presence of DA (N = 5)

(C) Effect of DA (N = 5)

RATS FED LPD FOR 2 DAYS

(A) Effect of PTH (N = 5)

(B) Effect of PTH in the presence of DA (N = 5)

(C) Effect of DA (N = 5)

RATS FED LPD FOR 3 DAYS

(A) Effect of PTH (N = 5)

(B) Effect of PTH in the presence of DA (N = 5)

(C) Effect of DA (N = 4)

Inulin concentrations in plasma and urine were measured by the anthrone method (14). Sodium and potassium concentrations in plasma and urine were measured with ion-selective electrodes (Beckman E2A analyzer; Beckman Instruments). Phosphate concentrations in plasma and urine were determined by the method of Chen et al. (13). The GFR was calculated on the basis of the clearance of inulin.

All values are reported as mean ± SE. One-way analysis of variance was used to compare the changes in fractional excretion of phosphate (FEp). If analysis of variance indicated overall group differences, then group comparisons were made by the Bonferroni method (15). Other comparisons were
RESULTS

In metabolic balance studies, urinary DA excretion was significantly lower during ingestion of a LPD than during ingestion of an HPD (2.53 ± 0.06 vs 4.10 ± 0.30 µg/day; see Table 1). By the first day of high-dietary-phosphate intake, urinary DA excretion was significantly increased from 2.50 ± 0.30 to 3.60 ± 0.20 µg/day. Urinary excretion of phosphate increased with a high dietary intake of phosphate, whereas no changes were observed in sodium excretion (Table 1).

In clearance studies, infusion of PTH in rats fed a NPD significantly increased the FEₚ from 4.3 ± 2.0% in the control clearance to 43.9 ± 3.4% in the experimental clearance. Animals fed a LPD exhibited a significantly and progressively blunted phosphaturic response to PTH as compared with rats fed a NPD. FEₚ increased from 1.0 ± 0.3 to 12.3 ± 2.9% in rats fed a LPD for 1 day; from 1.1 ± 0.6 to 6.2 ± 3.8% in rats fed a LPD for 2 days; and from 0.5 ± 0.2 to 0.3 ± 0.1% in rats fed a LPD for 3 days. No significant differences were found in the blunted phosphaturic response to PTH by rats fed a LPD for 1 as compared with 2 days. Rats fed a LPD for 3 days had a significantly lower response to PTH than did rats fed LPD for 1 day (Table 2; Figure 1).

Infusion of PTH in the presence of DA to rats fed a LPD resulted in a significantly enhanced phosphaturic response to PTH. FEₚ significantly increased from 1.0 ± 0.3 to 28.9 ± 5.8% in rats fed a LPD for 1 day; from 0.8 ± 0.2 to 29.2 ± 1.5% in rats fed a LPD for 2 days; and from 0.8 ± 0.3 to 11.5 ± 2.8% in rats fed a LPD for 3 days. Infusion of PTH in the presence of DA in rats fed a NPD resulted in a significant increase in FEₚ from 9.0 ± 2.3 to 34.4 ± 6.1% (Table 2; Figure 1).

The infusion of DA alone in rats fed a NPD significantly increased the FEₚ, but to lower levels than in response to PTH. FEₚ increased from 6.6 ± 1.3 to 16.2 ± 1.0%. In rats fed a LPD, the infusion of DA alone significantly increased the FEₚ up to the third day of the LPD. FEₚ increased from 1.6 ± 0.4 to 13.8 ± 4.6% in rats fed a LPD for 1 day and from 1.2 ± 0.7 to 12.0 ± 2.9% in rats fed a LPD for 2 days. Rats fed a LPD for 3 days and infused with DA showed no increase in the FEₚ (0.4 ± 0.3 to 0.3 ± 0.1%). No significant differences in the phosphaturic response to DA were found between rats fed a NPD and a LPD for 1 or 2 days. Rats fed a LPD for 3 days exhibited a significantly lower phosphaturic response to DA when compared with any of the other groups (Table 2; Figure 1).

Changes in FEₚ (ΔFEₚ) were greater in rats fed a NPD and infused with PTH alone (39.6 ± 4.5%) than in rats fed a NPD infused with PTH in the presence of DA (25.4 ± 3.4%) or DA alone (9.6 ± 2.2%). Rats fed a LPD and infused with PTH in the presence of DA significantly enhanced the ΔFEₚ, (27.9 ± 5.8% for day 1, 28.4 ± 1.4% for day 2, and 10.7 ± 2.8% for day 3) when compared with LPD rats infused with PTH alone (11.3 ± 2.6% for day 1, 7.1 ± 3.3% for day 2, and −0.2 ± 0.1% for day 3 (Figure 1).

Plasma phosphate decreased significantly after the experimental infusions only in rats fed a NPD infused with PTH (from 3.5 ± 0.1 to 2.6 ± 0.2 mM) and with PTH in the presence of DA (from 4.4 ± 0.3 to 3.5 ± 0.1 mM).

Fractional excretion of sodium significantly increased from control to experimental clearances in all animals infused with DA, except for the group of rats fed a LPD for 3 days infused with DA alone.

| Table 1. Effect of changes in dietary phosphate intake on urinary phosphate, sodium, and DA excretion |
|-----------------|-----------------|-----------------|-----------------|
| **LPD (N = 5)** | **Days** | **Vₑ** (mmol/day) | **Vₑₓ** (mEq/day) | **Vₑᵃ** (µg/day) |
| 1               | 0.23 ± 0.06    | 4.70 ± 0.40     | 2.50 ± 0.30     |
| 2               | 0.14 ± 0.06    | 5.60 ± 0.50     | 2.70 ± 0.30     |
| 3               | 0.05 ± 0.03    | 6.50 ± 0.30     | 2.40 ± 0.40     |
| 4               | 0               | 5.80 ± 0.30     | 2.50 ± 0.30     |
| Mean ± SE      | 0.11 ± 0.05b   | 5.67 ± 0.37     | 2.53 ± 0.06b    |
| **HPD (N = 5)** |                  |                  |                  |
| 1               | 2.80 ± 0.30    | 6.10 ± 0.50     | 3.60 ± 0.20     |
| 2               | 4.00 ± 0.20    | 5.20 ± 0.30     | 3.70 ± 0.30     |
| 3               | 4.80 ± 0.30    | 5.30 ± 0.30     | 4.70 ± 0.40     |
| 4               | 4.20 ± 0.10    | 5.20 ± 0.20     | 4.50 ± 0.40     |
| Mean ± SE      | 3.90 ± 0.40    | 5.40 ± 0.20     | 4.10 ± 0.30     |

*a Abbreviations: Vₑ, urinary excretion. Values are mean ± SE.
*b Significantly lower than HPD.
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<th>GFR (mL/min)</th>
<th>V (µl/min)</th>
<th>FE_{Na} (%)</th>
<th>FE_{P} (%)</th>
<th>P_{P} (mM)</th>
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<td>44 ± 5</td>
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<td>31 ± 10</td>
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<td>102 ± 19^p</td>
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<td>DA (N = 4)</td>
<td>2.1 ± 0.3</td>
<td>2.1 ± 0.2</td>
<td>46 ± 8</td>
<td>96 ± 13^p</td>
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^a Abbreviations: V, urinary flow rate; FE_{Na}, fractional excretion of sodium; P_{P}, plasma phosphate concentration; C, control; E, experimental.

^b Significant difference from control to experimental. Paired t test, P < 0.05.
significant increase in urinary flow rate was also observed in all of the animals infused with DA.

No significant difference was observed in GFR with any of the experimental infusions.

A significant increase in mean arterial pressure, from 106 ± 3 to 119 ± 3 mm Hg, was observed when rats infused with DA were combined.

**DISCUSSION**

The study presented here demonstrates that intrarenal synthesis of DA, as reflected by urinary DA excretion, was significantly lower in rats fed a LPD. Further, DA infusion significantly enhanced the phosphaturic response to PTH in short-term phosphate-deprived rats. This phosphaturic response (ΔFEp) was significantly greater than the respective change observed in rats fed a LPD for 1, 2, and 3 days and infused either with DA or PTH alone.

It is likely that DA plays a role in the early but not the late adaptation to phosphate deprivation. The renal adaptation to phosphate deprivation occurs very rapidly; detectable increases in phosphate uptake by brush border membranes have been demonstrated within 4 h of phosphate deprivation (16–18). Previous studies have suggested a role for the adrenergic nervous system in the short-term adaptation to phosphate deprivation. Acute infusion of propranolol (a β-adrenoreceptor antagonist) has been shown to restore the phosphaturic effect of PTH in short-term phosphate-deprived rats (19). Renal denervation has been reported to increase urinary DA excretion (20) and to restore the phosphaturic response to PTH in short-term phosphate-deprived rats (21). In the studies presented here, the effect of DA to enhance the phosphaturic response to PTH was most dramatic during the first and second day of phosphate deprivation, which suggests a role for DA in the short-term adaptation to phosphate deprivation.

It is interesting to note that DA was phosphaturic by itself, but to a lesser extent than was PTH, and that phosphate deprivation progressively blunted the phosphaturic response to PTH, whereas the phosphaturic response to DA was only blunted after the third day of a LPD. These findings, and the fact that DA markedly enhanced the phosphaturic response to PTH in rats fed a LPD for 1 and 2 days, suggest that the adaptation to phosphate deprivation in the presence of DA takes place later and that the phosphaturic effect of PTH may be modulated by DA during short-term phosphate deprivation. Thus, DA reverses the resistance to the phosphaturic effect of PTH in animals fed a LPD without affecting the response to PTH in rats fed a NPD. This suggests that DA reverses the influence of a LPD rather than primarily affecting the efficacy of PTH per se.

There is evidence to support the thesis that intrarenal production of DA may regulate phosphate reabsorption along the proximal tubule. DA is known to be phosphaturic (7) and to decrease the transport of phosphate by the proximal tubule (22). DA receptors have been identified on the proximal tubule, the major nephron site of phosphate reabsorption (23–26). DA has also been demonstrated to increase cAMP accumulation in renal tissue (27) and to inhibit the Na-K ATPase at the basolateral membrane in proximal tubular cells as the result of the activation of protein kinase C (28,29). Stimulation of phospholipase C by DA has been shown to be independent of the adenylate cyclase system in renal cortical homogenates (30). Further, recent studies from our laboratory have shown that DA significantly and specifically inhibited phosphate uptake by brush border membranes vesicles prepared from rat renal cortex (31). These findings suggest a selective effect of DA upon the rate-limiting step in proximal tubular phosphate reabsorption, i.e., the luminal Na-P, cotransporter (32,33). All of these mechanisms probably contribute to an increase in phosphate excretion and therefore support a role for DA in modulating the renal handling of phosphate.

It has been reported that physiological doses of PTH may regulate phosphate transport by the activation of protein kinase C, whereas higher concentrations appear to activate the adenylate cyclase system (34). DA has also been shown to activate both phospholipase C and adenylate cyclase in renal cortical plasma membrane preparations (27,29,30). Thus, because pharmacological doses of both hormones were used in this study, the adenylate cyclase system may have been sufficiently activated to provide an alternative mechanism to override the avid reabsorption of phosphate during short-term phosphate deprivation. However, mechanisms implicated in the resistance to the phosphaturic effect of PTH may occur at steps
beyond the accumulation of cAMP. It has been shown that infusion of the cAMP analog (8-4-chlorophenylthyo-adenosine 3,5-cyclic monophosphate) did not increase urinary phosphate excretion in phosphate-deprived rats (35).

In summary, animals fed a LPD conserve phosphate and exhibit a blunted phosphaturic response to PTH administration. In the study presented here, the infusion of DA significantly enhanced the phosphaturic response to PTH in rats fed a LPD for up to 3 days. Thus, low intrarenal DA synthesis may be a possible mechanism implicated in the resistance to the phosphaturic effect of PTH during short-term phosphate deprivation. Studies performed in conscious rats are consistent with this hypothesis, because urinary DA excretion was found to be significantly lower in rats fed a LPD than in those fed a HPD.

We conclude that during short-term phosphate deprivation, intrarenal DA synthesis is low and that DA infusion significantly enhances the phosphaturic response to PTH administration.

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