

# Increased renal dopamine and acute renal adaptation to a high-phosphate diet

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Departments of <sup>1</sup>Medicine and <sup>2</sup>Physiology, University of Maryland School of Medicine, and <sup>3</sup>Medical Service, Department of Veterans Affairs Medical Center, Baltimore, Maryland; <sup>4</sup>Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, Texas; <sup>5</sup>Department of Medicine, Yale University School of Medicine and Department of Veterans Affairs Connecticut Health Care System Medical Center, West Haven, Connecticut; and <sup>6</sup>Program in Neuroscience and Behavioral Disorders, Graduate Medical School, Duke-National University of Singapore, Singapore

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**Weinman EJ, Biswas R, Steplock D, Wang P, Lau Y, Desir GV, Shenolikar S.** Increased renal dopamine and acute renal adaptation to a high-phosphate diet. *Am J Physiol Renal Physiol* 300: F1123–F1129, 2011. First published February 16, 2011; doi:10.1152/ajprenal.00744.2010.—The current experiments explore the role of dopamine in facilitating the acute increase in renal phosphate excretion in response to a high-phosphate diet. Compared with a low-phosphate (0.1%) diet for 24 h, mice fed a high-phosphate (1.2%) diet had significantly higher rates of phosphate excretion in the urine associated with a two- to threefold increase in the dopamine content of the kidney and in the urinary excretion of dopamine. Animals fed a high-phosphate diet had a significant increase in the abundance and activity of renal DOPA (L-dihydroxyphenylalanine) decarboxylase and significant reductions in renal monoamine oxidase A and monoamine oxidase B. The activity of protein kinase A and protein kinase C, markers of activation of renal dopamine receptors, were significantly higher in animals fed a high-phosphate vs. a low-phosphate diet. Treatment of rats with carbidopa, an inhibitor of DOPA decarboxylase, impaired adaptation to a high-phosphate diet. These experiments indicate that the rapid adaptation to a high-phosphate diet involves alterations in key enzymes involved in dopamine synthesis and degradation, resulting in increased renal dopamine content and activation of the signaling cascade used by dopamine to inhibit the renal tubular reabsorption of phosphate.

renal phosphate transport; dopamine metabolism; mouse

IN RESPONSE TO INGESTION OF a high-phosphate diet, there is a rapid increase in the urinary excretion of phosphate that is manifest within hours and is associated with a decrease in the abundance of sodium-dependent phosphate transporters in the proximal tubule of the kidney (4, 16, 17, 23–25). By contrast, ingestion of a low-phosphate diet results in a rapid decrease in the urinary excretion of phosphate associated with recruitment of phosphate transporters to the apical membrane of renal proximal tubule cells. This adaptive response is considered to be a very important physiological mechanism mediating phosphate homeostasis and, although described several decades ago, an understanding of key elements of this physiological defense mechanism has remained elusive (4, 24). On one hand, the cells that sense the change in the dietary content of phosphate have been debated. Proximal tubule cells in culture incubated in high- or low-phosphate media reproduce many aspects of the adaptive response to changes in diet (8, 16, 20). These studies would suggest that proximal tubule cells can sense differences in the phosphate concentration of the media.

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It seems unlikely, however, that renal proximal tubule cells are sensors for changes in diet since changes in the dietary intake of phosphate do not result in changes in the serum concentration of phosphate. Kumar and colleagues (3, 14) have reported very important studies showing that perfusion of the duodenum with solutions containing high concentrations of phosphate results in a rapid increase in the urinary excretion of phosphate. While the mechanism by which the phosphate concentration is detected remains to be determined, these studies represent a significant advance in the understanding of the renal adaptive response to differences in the dietary intake of phosphate.

Another key element, and the focus of the current investigations, is how the kidney is signaled to regulate the renal tubular reabsorption of phosphate when the diet is altered. Evidence available to date would indicate that neither parathyroid hormone (PTH) nor fibroblast growth factor 23 (FGF-23) mediates the acute alterations in urinary phosphate excretion in response to changes in diet (3, 5, 11, 24). Recent studies have indicated that one of the major sodium-dependent phosphate transporters, Npt2a, is complexed in the apical membrane of proximal tubule cells to adaptor scaffolding proteins such as sodium-hydrogen exchanger regulatory factor-1 (NHERF-1) (13, 22). NHERF-1 binds 35–50% of the Npt2a in the apical membrane, and several defined regulatory processes such as the responses to PTH and to dopamine require the phosphorylation of NHERF-1 with a subsequent dissociation of NHERF-1/Npt2a complexes (26). We have previously reported that phosphate adaptation is abnormal in NHERF-1 null mice and absent in NHERF-1 null proximal tubule cells cultured in high- or low-phosphate media (8, 25). Given the role of NHERF-1 in the regulation of Npt2a and the fact that PTH is not required for the adaptive response, we hypothesized that dopamine participates in the regulation of the urinary excretion of phosphate in response to alterations in the dietary intake of phosphate.

## MATERIALS AND METHODS

### Animals and Diet Protocols

Male C57BL/6 mice age 12–16 wk and Sprague-Dawley rats, weighing 150–200 g, were housed in metabolic cages in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care international guidelines in the Baltimore Veterans Affairs Animal Care Facility. All animal experiments were approved by the University of Maryland, School of Medicine Animal Protocol Review Board. To determine the adaptive response to changes in the phosphate content of the diet in mice, the animals were stabilized on a normal rodent chow diet (phosphate content = 0.9%). Animals were then fed either a low-phosphate diet (0.1%) or a high-phosphate diet

(1.2%) for 24 hrs during which urine was collected for the determination of sodium, phosphate, creatinine, and dopamine concentrations. At the end of the urine collections, the kidneys were harvested for measurement of dopamine content. In the studies involving rats, all animals were stabilized on the low-phosphate diet for 24 h and then changed to the high-phosphate diet for 24 h. Animals received intraperitoneal injections of carbidopa or the methanol diluent (controls) once/day for both days of the study. Urine was collected daily, and the kidneys were harvested at the end of the high-phosphate diet collection period.

### Biochemical Assays

**Dopamine.** Kidneys were harvested, and the capsule was stripped, weighed, and homogenized in ice-cold 0.2 N perchloric acid with a handheld polytron for 15 min. Each sample was centrifuged at 11,000 g for 15 min at 4°C, and the supernatant was collected. The urine samples were suspended in 0.01% glutathione and 0.05% Na<sub>2</sub>EDTA. Each kidney lysate or urine sample was spiked with an internal standard, epinine, and extracted with alumina, which had been equilibrated with a buffer consisting of 1.5 M Tris-HCl and 1% Na<sub>2</sub>EDTA (pH 8.6). The sample-alumina mixture was precipitated by centrifugation (11,000 g, 4°C), followed by washing twice with distilled/deionized water. The alumina-extracted sample was eluted with 0.2 ml of 0.2 N perchloric acid and filtered through a nylon syringe filter (Gelman ACRO LC3A, 0.45 mm). An aliquot of the filtrate was injected into HPLC (model 1525, Waters, Milford, MA) equipped with a C<sub>18</sub> reverse-phase, 3- $\mu$ m LUNA column (100  $\times$  2.0 mm, Phenomenex, Torrance, CA). The sample was eluted by a mobile phase made of 25 mM NaH<sub>2</sub>PO<sub>4</sub>, 50 mM Na-citrate, 0.03 mM EDTA, 10 mM diethylamine HCl, and 2.2 mM sodium octyl sulfate (pH 3.2), 30 ml/l methanol, and 22 ml/l dimethylacetamide at a flow rate of 0.4 ml/min. Dopamine contents were determined by the Coulometric electrochemical detector (model Coulochem III, ESA, Chelmsford, MA), calculated from the known standard curve, and corrected for sample loss due to extraction based on the recovery of the internal standard, epinine (15).

**Renalase.** The kidneys were harvested as above and suspended in 2 ml of an ice-cold buffer containing 10% glycerol, 10 mM NaCl, 1 mM EDTA, 0.5 mM DTT, 25 mM BisTris, pH 6.5, and one Complete Protease Inhibitor Cocktail table (Roche). The kidneys were homogenized using a hand-held polytron, the tissue was centrifuged 4°C at 14,000 rpm for 15 min, and the supernatants were used for assay of renalase activity. Renalase is a FAD-dependent oxidoreductase, which uses NADH as a cofactor to reduce its FAD moiety. In the presence of oxygen, the reduced renalase-FADH complex metabolizes substrates such as catecholamines (27). Renalase has intrinsic NADH oxidase activity, and in the absence of substrates, can convert NADH to NAD<sup>+</sup> and generate superoxide anion as a byproduct. The assay buffer contained 25 mM Tris, pH 7.5, and 5 mM NaCl. NADH was made fresh and added to a final concentration of 200  $\mu$ M. The reaction was initiated by adding 70  $\mu$ g of kidney lysate to 200  $\mu$ l ml of assay buffer in 96-well plate cuvettes (0.6-cm path length). Seventy micrograms of BSA served as a negative control. Absorbance at 340 nm was measured in a plate reader at 37°C every 4 min for up to 60 min. The amount of NADH oxidized to NAD was calculated from the decrease in absorbance at 340 nm using a molar extinction coefficient of 0.00622  $\mu$ M<sup>-1</sup>·cm<sup>-1</sup>. Background corrections were made by subtracting the changes in absorbance obtained with BSA.

**DOPA decarboxylase.** Kidney lysates were prepared as in the renalase assay and incubated in a solution containing 20 mM Tris (pH 7.2), 0.1 mM pyridoxal phosphate, 0.1 mM EDTA, 2 mM dithiothreitol, 1.30 mM L-DOPA, and 0.03 mM DOPA, DL-3,4-alanine 1-<sup>14</sup>C-DOPA at 37°C for 30 min in closed reaction tubes containing two discs of Whatman paper in 1 M benzethonium hydroxide. The reaction was stopped by the addition of 0.5 ml of 2 M citric acid. The

discs were then transferred to scintillation vials containing 10 ml of scintillation fluid and counted (18).

**Monoamine oxidase A and B.** Kidney tissue was homogenized in a Tris buffer containing 10% glycerol, 10 mM NaCl, 1 mM EDTA, 0.5 mM DTT, 25 mM BisTris, pH 6.5, and one Roche protease inhibitor tablet. Monoamine oxidase A and monoamine oxidase B were assayed using the Amplex red monoamine oxidase (MAO-A/MAO-B) assay kit. The assay detects the generation of hydrogen peroxide in a horseradish peroxidase-coupled reaction using Amplex red reagent (10-acetyl-3,7-dihydroxyphenoxazine). The fluorescence of resorufin, the reaction product, was determined at an excitation range of 530–560 nm, and the emission was detected at 590 nm. To facilitate the discrimination of the two enzymes, assays were performed using *p*-tyramine, a substrate for both enzymes; benzylamine, a substrate for monoamine oxidase B; clorgyline, a specific inhibitor of monoamine oxidase A; and pargyline, a specific inhibitor of monoamine oxidase B as described by the manufacturer.

**Catechol-O-methyl transferase.** Kidney lysates were prepared as in the monoamine oxidase assays. Catechol-O-methyl transferase is a soluble magnesium requiring a cytoplasmic enzyme that catalyzes the transfer of a methyl group from S-adenosyl-L-methionine to substrates containing the catechol moiety. Fifty microliters of kidney lysate was mixed with 500  $\mu$ l of a freshly prepared buffer-substrate mixture containing 400  $\mu$ l potassium phosphate buffer (0.1 M, pH 7.6), 20  $\mu$ l magnesium chloride (0.1 M), 30  $\mu$ l catechol (1,2-dihydroxybenzene, 0.05 M), 20  $\mu$ l of DTT (0.065 M), 10  $\mu$ l of adenosine deaminase (470 U/ml), and 2  $\mu$ l of [<sup>3</sup>H]Ado-Met (5.5 M, specific activity 3.64 Ci/mol). The reaction was initiated by placing the tubes in a shaking water bath at 37°C. After 30 min, the reaction was stopped by immersing the tubes in an ice bath. Five hundred microliters of 1 N HCl containing 1 g/l guaiacol was added, and the mixture was transferred to 10 ml of scintillation fluid. The vials were capped, the [<sup>3</sup>H]guaiacol was extracted into the upper phase by vigorous shaking for 1 min, and the sample was counted in a scintillation counter (28).

**PKA and PKC.** PKA and PKC activity was determined as previously described using a cAMP-Dependent Protein Kinase (PKA) Radioactive Assay Kit, (SignaTECT, Promega) and Promega's SignaTECT PKC Assay System containing a specific PKC substrate and capture membrane.

### Other Methods

Western immunoblots were performed on kidney lysates using commercially available antibodies to tyrosine hydroxylase, DOPA decarboxylase, monoamine oxidase A, monoamine oxidase B, catechol-O-methyl transferase, and renalase. Equality of loading was determined using Ponceau S staining or ezrin antibodies as previously described (25). Small corrections were applied for minor differences in loading. Protein concentrations were determined using the method of Lowry et al. (19).

### Statistical Analyses

Statistical analyses were performed using Peritz ANOVA. *P* values <0.05 were considered statistically significant.

## RESULTS

The urine phosphate-to-creatinine ratio averaged  $30.7 \pm 2.6$  and  $0.5 \pm 0.2$  ( $n = 6$ ,  $P < 0.05$ ) in animals fed a high- (1.2%) or low- (0.1%) phosphate diet, respectively. The urine sodium-to-creatinine ratio, as a surrogate for consumption of the diet, was  $7.7 \pm 0.3$  in animals fed the high-phosphate diet and  $7.3 \pm 0.2$  in animals fed the low-phosphate diet ( $n = 6$ ,  $P =$  not significant). Renal tissue dopamine concentrations were significantly higher in animals fed the high-phosphate diet ( $1.31 \pm 0.31$  ng/mg of tissue) compared with animals fed the low-

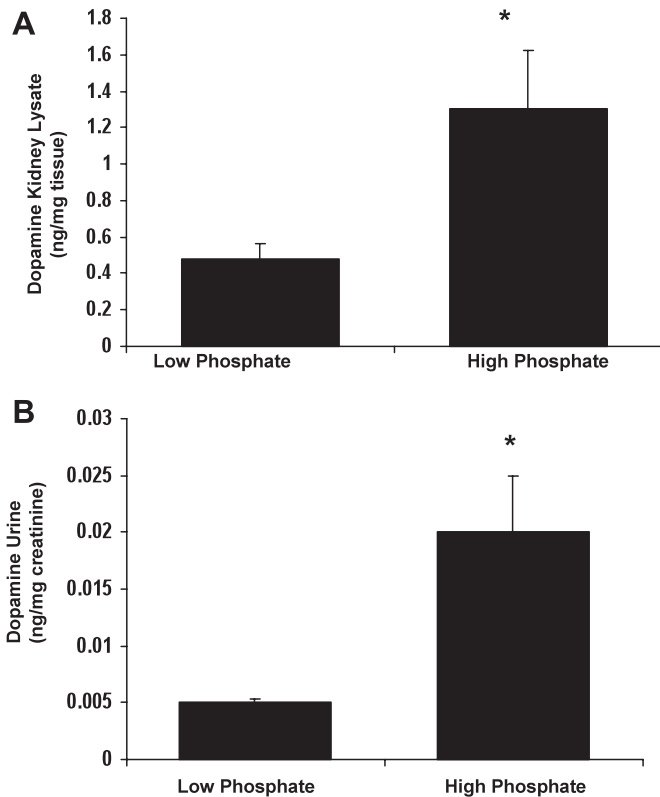


Fig. 1. Dopamine concentrations in kidney and urine of mice fed a low-phosphate or high-phosphate diet for 24 h. *A*: dopamine concentrations in kidney lysates (means  $\pm$  SE). *B*: urine dopamine concentrations (means  $\pm$  SE).  $P < 0.05$ .

phosphate diet ( $0.49 \pm 0.07$ ,  $n = 3$ ,  $P < 0.05$ ) (Fig. 1*A*). The urinary excretion of dopamine was also higher in mice fed the high-phosphate diet ( $0.019 \pm 0.003$  ng/mg creatinine) vs. the low-phosphate diet ( $0.005 \pm 0.002$ ,  $n = 3$ ,  $P < 0.05$ ) (Fig. 1*B*).

To examine the mechanism of the increased renal and urine concentrations of dopamine, we measured the abundance and/or activity of several of the key metabolic enzymes involved in dopamine biosynthesis or degradation. In kidney lysates, the abundance of tyrosine hydroxylase did not differ between animals fed the high-phosphate diet ( $0.17 \pm 0.02$  arbitrary units) or the low-phosphate diet ( $0.14 \pm 0.03$ ,  $n = 3$ ,  $P =$  not significant). By contrast, the abundance of DOPA decarboxylase was significantly higher in animals fed a diet high in phosphate ( $10.8 \pm 1.6$  arbitrary units) compared with animals fed the low-phosphate diet ( $3.6 \pm 0.4$ ,  $n = 4$ ,  $P < 0.05$ ) (Fig. 2, *A* and *B*). DOPA decarboxylase activity was significantly higher in animals fed a diet high in phosphate ( $89.3 \pm 9.3$  nmol  $\text{CO}_2 \cdot \text{min}^{-1} \cdot \mu\text{g protein}^{-1}$ ) vs. the low-phosphate diet ( $39.8 \pm 4.6$ ,  $n = 6$ ,  $P < 0.05$ ) (Fig. 2*C*). We next assayed the abundance and activity of enzymes involved in dopamine degradation. The abundance of renalase in kidney lysates was significantly lower in animals fed a diet high in phosphate ( $1.1 \pm 0.3$  arbitrary units) compared with animals fed the low-phosphate diet ( $3.0 \pm 0.8$ ,  $n = 4$ ,  $P < 0.05$ ) (Fig. 3, *A* and *B*). As shown in Fig. 3*C*, renalase activity was significantly lower in animals fed a diet high in phosphate vs. animals fed the low-phosphate diet ( $n = 6$ ). The abundance and activity of renal monoamine oxidase A and monoamine oxidase B were

also significantly lower in animals fed a diet high in phosphate compared with animals fed the low-phosphate diet (Figs. 4, *A–C*, and 5, *A–C*). On the other hand, neither the abundance nor activity of renal catechol-O-methyl transferase was different between mice fed a high- compared with a low-phosphate diet (Fig. 6, *A–C*).

To determine whether dopamine-related downstream signaling pathways were activated, we measured PKA and PKC activity in kidney lysates from animals fed the high- or low-phosphate diet. PKA activity was  $2.2 \pm 0.2$ -fold higher in the high-phosphate diet-fed animals compared with the animals fed the low-phosphate diet ( $n = 6$ ,  $P < 0.05$ ) (Fig. 7*A*). PKC activity was also significantly higher in the high- as compared low-phosphate-fed animals ( $2.4 \pm 0.2$ -fold,  $n = 6$ ,  $P < 0.05$ ) (Fig. 7*B*).

We then sought to examine the biological significance of the elevated renal concentration of dopamine. The intraperitoneal injection of carbidopa in concentrations from 40 to 480  $\mu\text{mol/kg}$  body wt was associated with a significant rate of death in both experimental and control mice, likely due to the volume of methanol required to solubilize carbidopa. Accord-

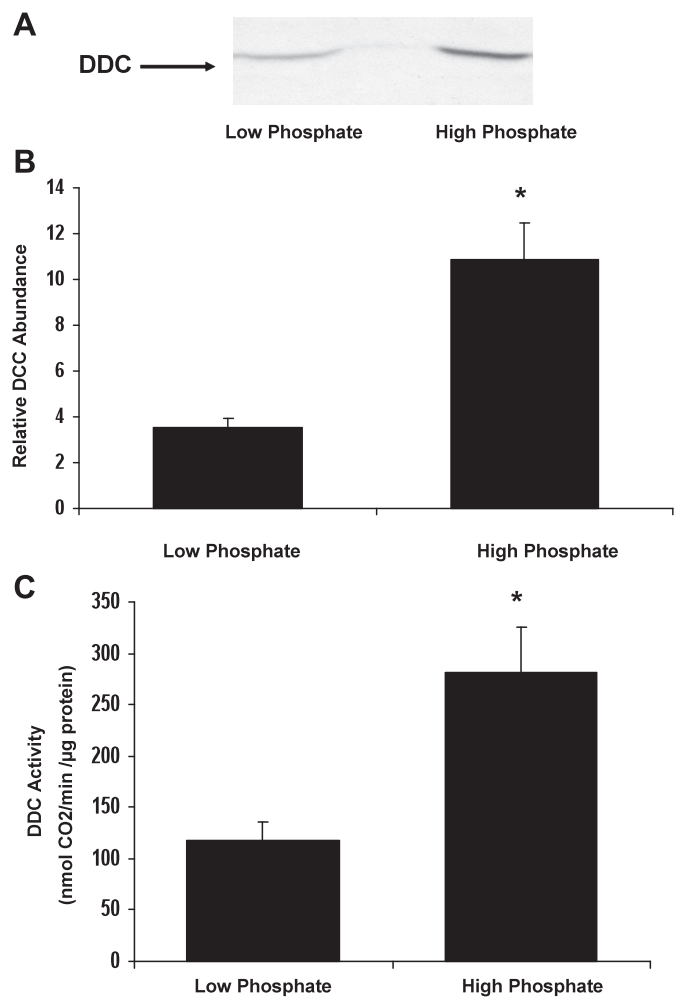


Fig. 2. Abundance and activity of DOPA decarboxylase (DDC) in the kidney of mice fed a low-phosphate or high-phosphate diet for 24 h. *A*: representative Western immunoblot. *B*: summary of relative abundance of DDC (means  $\pm$  SE;  $n = 4$ ). *C*: DDC activity expressed as nmol  $\text{CO}_2 \cdot \text{min}^{-1} \cdot \mu\text{g protein}^{-1}$  (means  $\pm$  SE;  $n = 4$ ).  $*P < 0.05$ .



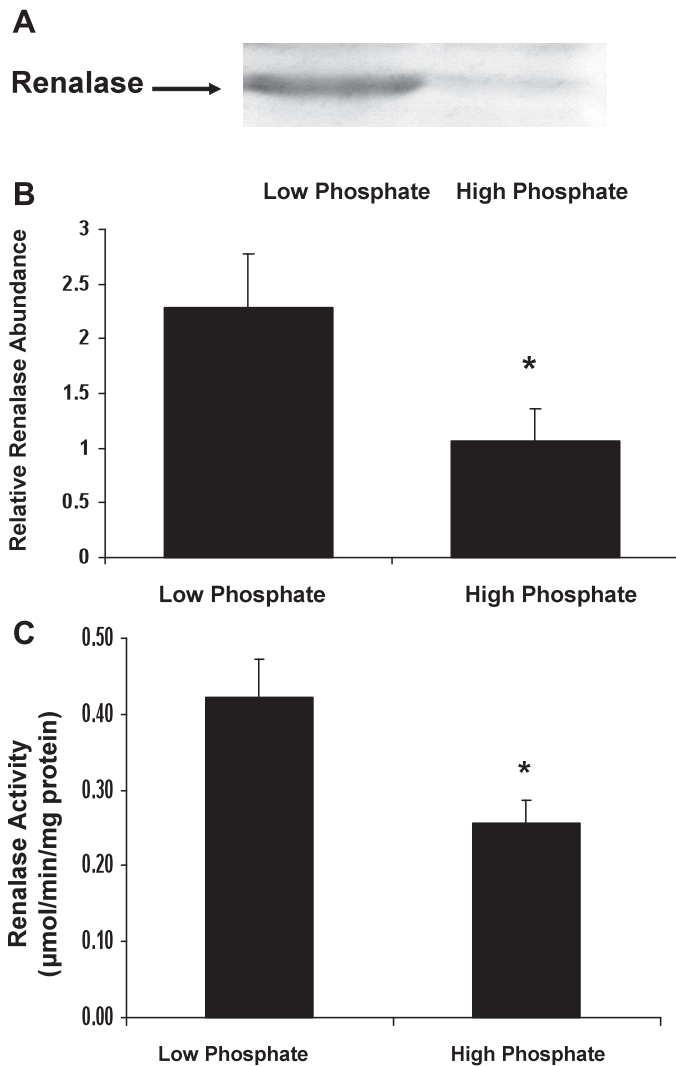


Fig. 3. Abundance and activity of renalase in the kidney of mice fed a low-phosphate or high-phosphate diet for 24 h. A: representative Western immunoblot. B: summary of relative abundance of renalase (means ± SE; n = 6). C: renalase activity expressed as NADH oxidation (nmol/mg protein; means ± SE; n = 6). \*P < 0.05.

ingly, we altered the protocol and repeated the studies in rats where prior published results suggested the drugs would be better tolerated (9). Rats were fed the low-phosphate diet for 24 h and then changed to the high-phosphate diet for 24 h. Intraperitoneal injection of methanol with and without carbidopa (240 μmol/kg body wt) was administered daily while animals were on both the low- and the high-phosphate diet. Urine was collected throughout the study periods, and the kidneys were harvested after completion of the urine collection in animals on the high-phosphate diet. As summarized in Table 1, while on the high phosphate diet, the urine phosphate-to-creatinine ratio was significantly lower in rats injected with carbidopa (4.3 ± 0.8) compared with control animals (7.7 ± 0.3, n = 6, P > 0.05). This was associated with a lower renal tissue concentration of dopamine of 0.49 ± 0.12 ng/mg tissue in control animals compared with 0.18 ± 0.03 (n = 6, P < 0.05) in animals treated with carbidopa.

DISCUSSION

The capacity of the kidney to adjust the rates of excretion of phosphate in response to changes in dietary intake is considered to be a very important homeostatic mechanism to regulate tissue and serum concentrations of inorganic phosphate (4, 16, 17, 20, 23–25). Recent studies have indicated that the cells of the duodenum sense the changes in diet and signal the kidney to alter the rates of phosphate excretion (3). The mechanism(s) that regulates the rate of excretion by the kidney in response to alterations in the dietary intake of phosphate, however, have remained enigmatic. Parathyroid hormone levels are not altered acutely in response to changes in the intake of phosphate, and the adaptive response is normal in thyroparathyroidectomized animals (3, 24). More recent experiments also tend to exclude a role for FGF-23 and vitamin D in the rapid adaptation to changes in the dietary intake of phosphate (3). Given the

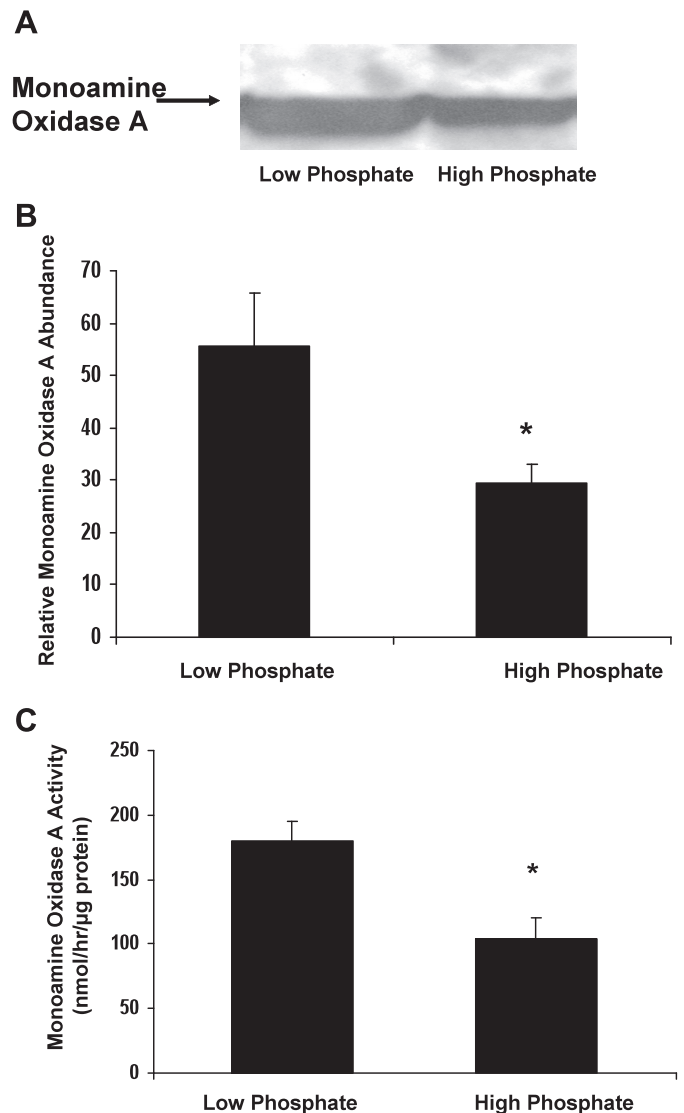


Fig. 4. Abundance and activity of monoamine oxidase A in the kidney of mice fed a low-phosphate or high-phosphate diet for 24 h. A: representative Western immunoblot. B: summary of relative abundance of monoamine oxidase A (means ± SE; n = 5). C: monoamine oxidase A activity expressed as nmol·h<sup>-1</sup>·μg protein<sup>-1</sup> (means ± SE; n = 5). \*P < 0.05.

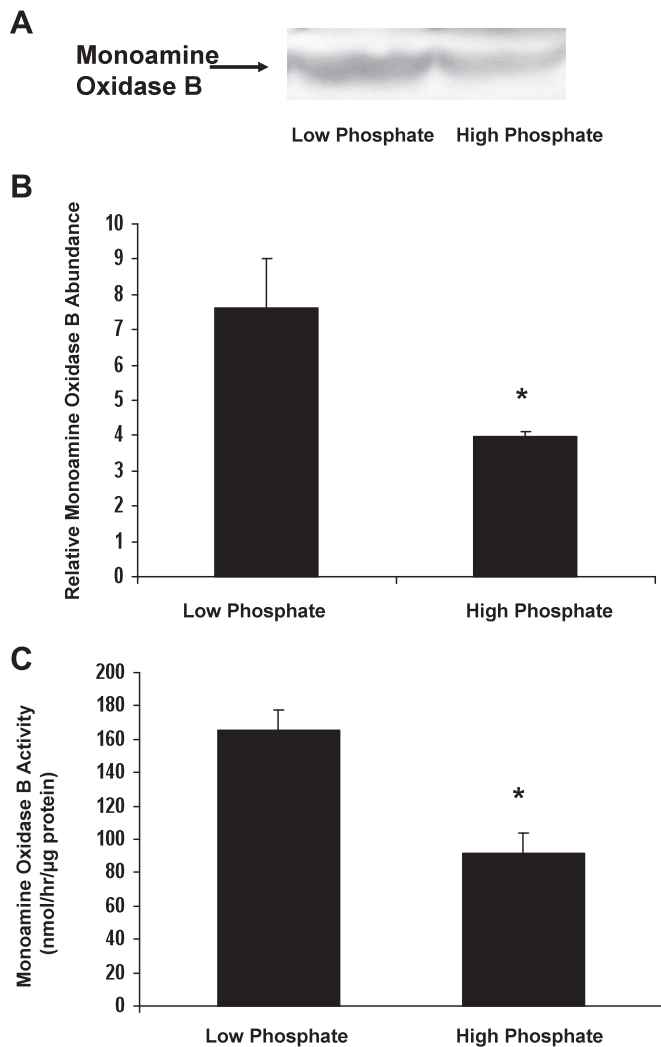


Fig. 5. Abundance and activity of monoamine oxidase B in the kidney of mice fed a low-phosphate or high-phosphate diet for 24 h. *A*: representative Western immunoblot. *B*: summary of relative abundance of monoamine oxidase B (means  $\pm$  SE;  $n = 4$ ). *C*: monoamine oxidase A activity expressed as  $\text{nmol}\cdot\text{h}^{-1}\cdot\mu\text{g protein}^{-1}$  (means  $\pm$  SE;  $n = 4$ ). \* $P < 0.05$ .

exclusion of these major regulators of the urinary excretion of phosphate, we designed experiments to explore the potential role of endogenous dopamine in the regulation of the urinary excretion of phosphate in response to alterations in the dietary intake of phosphate. The renal content of dopamine as well as the urinary excretion of dopamine was about two to three times higher in animals on a high- compared with a low-phosphate diet for 24 h. To understand the mechanism by which dopamine concentrations were increased, we assayed several of the enzymes involved in dopamine metabolism. Tyrosine hydroxylase abundance in the kidney was not different in mice ingesting a high- vs. a low-phosphate diet. DOPA decarboxylase abundance and activity, on the other hand, was significantly higher in high vs. low phosphate-fed animals. We then assayed the abundance and activity of renalase, a FAD-dependent oxidoreductase that metabolizes a variety of substrates including dopamine, as well as monoamine oxidase A, monoamine oxidase B, and catechol-*O*-methyl transferase (27). The renal abundance and activity of renalase, monoamine oxidase

A, and monoamine oxidase B were significantly lower in mice fed a high- compared with a low-phosphate diet. In most tissues, the activity of tyrosine hydroxylase rather than DOPA decarboxylase is considered to be rate limiting for dopamine biosynthesis. While tyrosine hydroxylase has been detected in the renal vasculature, it has not unequivocally been detected in renal proximal tubule cells (6). Nonetheless, renal proximal tubule cells can reabsorb L-dopa, and, under these circumstances, the activity of DOPA decarboxylase would be predicted to have an important influence on dopamine production (7). In addition, in response to a high-phosphate diet, the activity of three of the major dopamine-degrading enzymes, renalase, monoamine oxidase A, and monoamine oxidase B, are decreased. It would appear, then, that the integrated response to a high-phosphate diet involves increased synthesis as well as decreased catabolism of dopamine. In this regard, it is of interest that Knox and Dousa (1, 2) had previously reported an increase in the urinary excretion of dopamine in rats fed a high-phosphate diet. Recent studies have indicated that dopamine-mediated inhibition of phosphate transport involves activation of dopamine D1-like receptors and signaling through

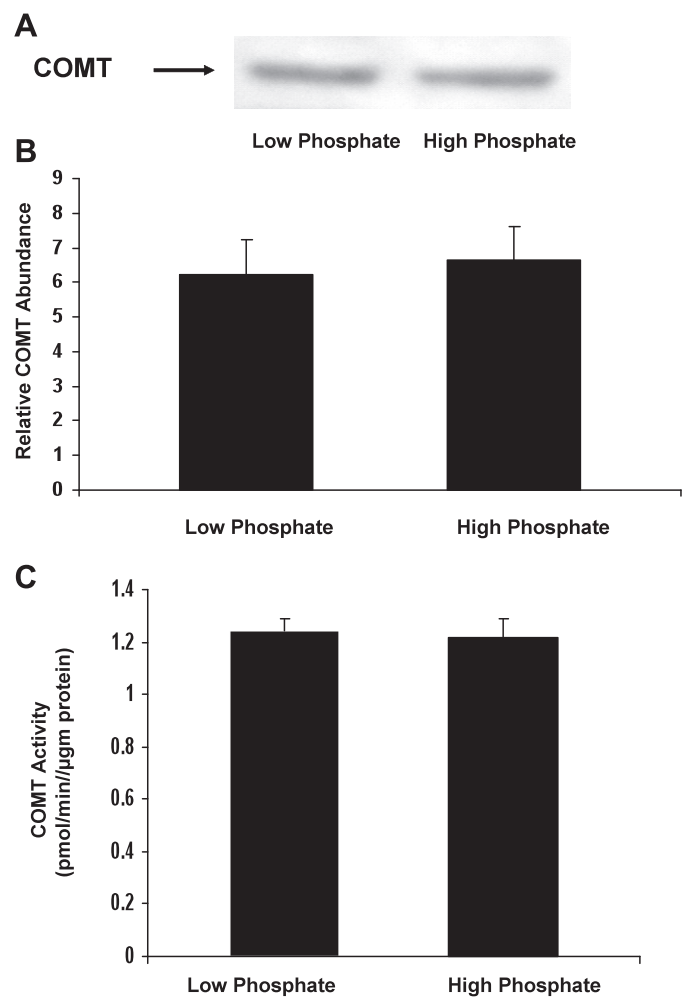


Fig. 6. Abundance and activity of catechol *O*-methyl transferase (COMT) in the kidney of mice fed a low-phosphate or high-phosphate diet for 24 h. *A*: representative Western immunoblot. *B*: summary of relative abundance of COMT (means  $\pm$  SE;  $n = 5$ ). *C*: COMT activity expressed as  $\text{pmol}\cdot\text{min}^{-1}\cdot\mu\text{g protein}^{-1}$  (means  $\pm$  SE;  $n = 5$ ). \* $P < 0.05$ .

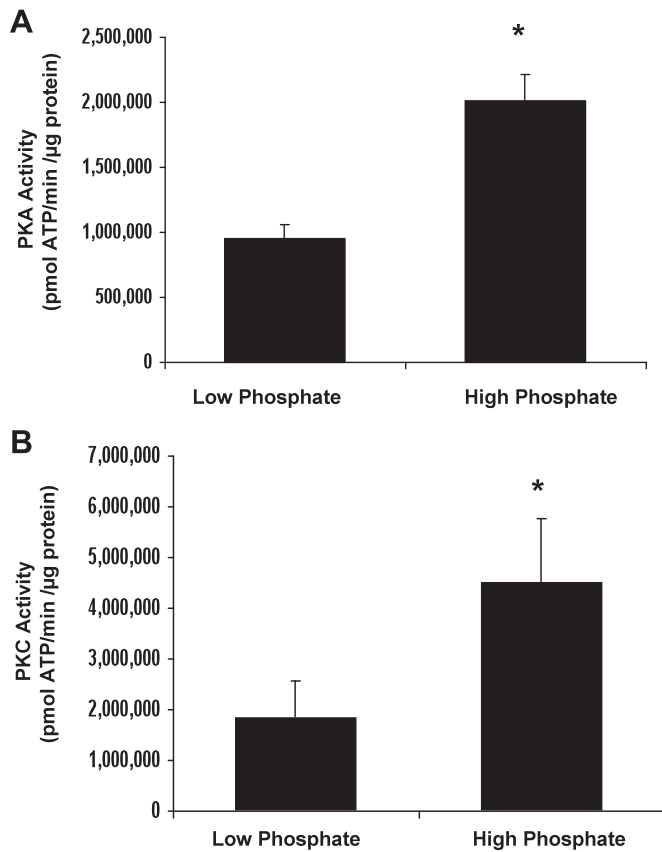


Fig. 7. PKA and PKC activity in the kidney of mice fed a low-phosphate or high-phosphate diet for 24 h. A: PKA activity (means  $\pm$  SE;  $n = 6$ ). B: PKC activity (means  $\pm$  SE;  $n = 6$ ). \* $P < 0.05$ .

activation of both PKA and PKC (9). Compared with ingestion of a low-phosphate diet, ingestion of a high-phosphate diet was associated with increased PKA and PKC activity in the kidney, suggesting an appropriate renal response to the increased tissue concentration of dopamine. Finally, we wished to demonstrate the importance of dopamine in the adaptive response to high-phosphate diets. In mice, intraperitoneal injections of carbidopa, an inhibitor of DOPA decarboxylase, dissolved in methanol or methanol alone was associated with high death rates in both groups. Accordingly, we elected to study rats who adapt to alterations in the phosphate content of the diet in a manner similar to mice and in whom the urinary excretion of dopamine is increased in response to a high-phosphate diet (1, 2). Our results indicate a dose of carbidopa of 240  $\mu\text{mol/kg}$  body wt, a dose higher than that used by Baines and colleagues (10), was well tolerated and effective in decreasing the urinary excretion of dopamine and blunting the adaptive response to the feeding of a high-phosphate diet. This suggests that the renal concentration of dopamine is relevant to how animals regulate the urinary excretion of phosphate in response to alterations in the dietary intake of phosphate.

The results of the current experiments are also of interest from another perspective. Dopamine has been well documented to affect the activity of a number of transporters in the renal proximal tubule, including Npt2a (9). Most of these studies have involved incubation of cultured cells with dopamine or the systemic infusion of dopamine. Such studies,

however, do not address the important issue related to the role of endogenous dopamine in the regulation of renal phosphate excretion. Only a limited number of prior studies have attempted to address this question. For example, the previously cited experiments of Baines and colleagues (10) in rats indicated that inhibition of DOPA decarboxylase resulted in an increase in renal phosphate reabsorption associated with decreased urinary excretion of dopamine. These and other investigators have suggested that dopamine may act in an autocrine/paracrine manner to regulate phosphate excretion in the urine (10, 12). Other studies, however, have suggested that regulation of endogenous dopamine production or degradation does not play a significant role in the regulation of phosphate excretion by the kidney (21). In this context, the present studies provide supportive evidence that regulation of the concentration of dopamine in the kidney plays a role in modulating important physiological processes such as the ability to alter the rates of phosphate excretion when the dietary intake of phosphate is changed.

In summary, the results of the present experiments indicate that the renal concentration of dopamine is increased in animals fed a high- compared with a low-phosphate diet. The increase in renal dopamine content is the result of alterations in the abundance and activity of several of the enzymes involved in dopamine metabolism. Inhibition of renal dopamine production results in a decreased capacity to adapt to a diet high in phosphate. In the aggregate, these studies provide evidence that regulation of endogenous dopamine plays an important biological role in regulating phosphate balance. There remain, however, a number of intriguing questions. First, it is not known how alterations in the dietary intake of phosphate signal the kidney to alter the activity of the enzymes involved in dopamine production and degradation. Kumar (14) has reported preliminary evidence that extracts of duodenum increase the urinary excretion of phosphate and have suggested that these extracts contain an as yet unidentified phosphaturic factor. At the present time, however, it is unknown how this putative factor might relate to our observations regarding the role of dopamine in the adaptive response to alterations in the dietary intake of phosphate. Second, the current experiments examined only the response to 24 h on a high- or low-phosphate diet, and

Table 1. Effect of carbidopa on phosphate and dopamine excretion in the rat

Group	Low-Phosphate Diet	High-Phosphate Diet
Control		
Urine $\text{PO}_4/\text{Cr}$	0.23 $\pm$ 0.16	7.7 $\pm$ 0.3
Urine $\text{Na}/\text{Cr}$	2.2 $\pm$ 0.2	1.9 $\pm$ 0.8
Renal dopamine, ng/mg tissue		0.49 $\pm$ 0.12
Carbidopa		
Urine $\text{PO}_4/\text{Cr}$	0.07 $\pm$ 0.03	4.3 $\pm$ 0.8*
Urine $\text{Na}/\text{Cr}$	2.3 $\pm$ 0.4	2.2 $\pm$ 0.2
Renal dopamine, ng/mg tissue		0.18 $\pm$ 0.03*

Values are means  $\pm$  SE for 6 animals/group. Rats were fed a low-phosphate diet for 24 h followed by a high-phosphate diet for 24 h. Urine was collected for measurement of phosphate, sodium, and creatinine (Cr) concentrations during both periods. At the end of the 24 h on the high-phosphate diet, the kidneys were harvested for measurement of dopamine content. Experimental animals received carbidopa (240  $\mu\text{mol/kg}$  body wt ip) on both days of the study, while control rats receive comparable volumes of methanol, the diluent for carbidopa. \* $P < 0.05$ .

it is not certain that the increase in the renal concentration of dopamine is the only operative mechanism. G protein-coupled receptors such as the dopamine receptors are known to undergo desensitization with prolonged exposure to their agonists. At 24 h, the activity of both PKA and PKC is increased. The adaptive responses to changes in the dietary intake of phosphate, however, are known to persist as long as the specific diet is continued. Thus it seems to us unlikely that the effect of dopamine would persist unabated, suggesting that other mechanisms may become operative with longer periods of dietary phosphate adaptation. We would suggest, therefore, that defense of plasma phosphate concentrations when the dietary intake of phosphate is increased requires the coordinated effects of multiple factors that include an increase in dopamine acutely followed by a rise in other factors such as FGF-23, in the longer term (3, 6, 11).

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#### DISCLOSURES

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