High dietary phosphate intake induces hypertension and augments exercise pressor reflex function in rats

Masaki Mizuno,1,2 Jere H. Mitchell,2 Scott Crawford,1 Chou-Long Huang,3,6 Naim Maalouf,5,6 Ming-Chang Hu,5,6 Orson W. Moe,3,6 Scott A. Smith,1,2 and Wanpen Vongpatanasin2,4,6

1Department of Health Care Sciences, Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, Texas; 2Cardiology Division, University of Texas Southwestern Medical Center, Dallas, Texas; 3Nephrology Division, University of Texas Southwestern Medical Center, Dallas, Texas; 4Hypertension Section, University of Texas Southwestern Medical Center, Dallas, Texas; 5Mineral Metabolism Division, University of Texas Southwestern Medical Center, Dallas, Texas; and 6Charles and Jane Pak Center of Mineral Metabolism and Clinical Research, University of Texas Southwestern Medical Center, Dallas, Texas

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INORGANIC PHOSPHATES ARE WIDELY used in the food industry as preservatives, flavor enhancers, and color stabilizers (1, 46). As a result, dietary phosphate (Pi) intake in the United States far exceeds the recommended daily allowance (1). High Pi intake is proposed to contribute to vascular calcification and cardiovascular mortality in patients with chronic kidney disease (18). However, little attention has been paid to the potential role of Pi excess on the cardiovascular system in the general population with normal kidney function. A high-Pi diet was recently shown to trigger blood pressure (BP) elevation in both normotensive rats (2) and spontaneously hypertensive rats (48), both with normal kidney function, in the resting condition. However, the influence of dietary Pi on BP regulation during physical exertion has not been determined.

Studies have demonstrated a correlation between dietary Pi intake and Pi concentration in the brain combined with the central nervous system’s role in regulating EPR-mediated SNA and BP response to exercise. Accordingly, we measured renal SNA (RSNA) and mean BP (MBP) in normotensive Sprague-Dawley rats fed a normal Pi diet (0.6%, n = 13) or high Pi diet (1.2%, n = 13) for 3 mo. As previously reported, we found that resting BP was significantly increased by 1.2% Pi diet in both conscious and anesthetized animals. Activation of the EPR by electrically induced hindlimb contraction triggered greater increases in δRSNA and δMBP in the 1.2% compared with 0.6% Pi group (126 ± 25 vs. 42 ± 9; 44 ± 5 vs. 14 ± 2 mmHg, respectively, P < 0.01). Activation of the muscle mechanoreflex, a component of the EPR, by passively stretching hindlimb muscle also evoked greater increases in δRSNA and δMBP in the 1.2% compared with 0.6% Pi group (109 ± 27 vs. 24 ± 7, 38 ± 7 vs. 8 ± 2 mmHg, respectively, P < 0.01). A similar response was produced by hindlimb intra-arterial capsaicin administration to stimulate the metaboreflex arm of the EPR. Thus, our data demonstrate a novel action of dietary Pi loading in augmenting EPR function through overactivation of both the muscle mechanoreflex and metaboreflex.

phosphate; exercise pressor reflex; blood pressure; sympathetic nerve activity; hypertension; diet; Western diet

Address for reprint requests and other correspondence: W. Vongpatanasin, Hypertension Section, Cardiology Division, Univ. of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., U9.400, Dallas, TX 75390-8586 (e-mail: wanpen.vongpatanasin@utsouthwestern.edu).

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of the right hindlimb were isolated. The calcaneal bone of the right hindlimb was cut and the Achilles tendon connected to a force transducer for the measurement of muscle tension. To activate the EPR, the gastrocnemius and soleus muscles were contracted by electrically stimulating the L4 and L5 ventral roots for 30 s using constant current stimulation at 3 times motor threshold with a pulse duration of 0.1 ms at 40 Hz.

Procedures to activate the mechanically sensitive component of the EPR. To evoke a mechanical stimulus similar to that elicited during muscle contraction, the gastrocnemius and soleus muscles of the right hindlimb were passively stretched for 30 s using a rack-and-pinion system (30, 32). Care was taken to manually generate the same pattern, as well as the magnitude of muscle tension developed during static muscle contractions. This maneuver was used to selectively activate the mechanoreceptors associated with the mechanically sensitive afferent fibers of the skeletal muscle mechanoreflex.

Procedures to activate the chemically sensitive component of the EPR. Selective activation of chemically sensitive metaboreceptors and associated skeletal muscle afferent fibers was achieved by injecting capsaicin into the arterial supply of the right hindlimb (0.3 μg/100 μl). Capsaicin was administered into the right common iliac artery, while a reversible ligature placed around the right common iliac vein was occluded for 2 min. This maneuver was used to selectively activate the afferent fibers associated with the skeletal muscle metaboreflex (30, 32).

End experiment procedures. To validate that RSNA signals were recorded from postganglionic renal sympathetic fibers, an intravenous infusion of hexamethonium bromide (60 mg/kg) was given to abolish SNA at the end of experiments. RSNA background noise was determined over a 30-min period after the insentient decerebrated animal.
was humanely killed by intravenous injection of saturated potassium chloride (4 M, 2 ml/kg). The heart and lungs were excised and weighed. Further, tibial length was measured.

Data Acquisition

Mean arterial pressure (MAP), HR, RSNA, and contractile tension data were acquired, recorded, and analyzed using data acquisition software (LabChart, ADInstruments) for the PowerLab analog-to-digital convertor (PowerLab8/30, ADInstruments) at a 1-kHz sampling rate. To analyze RSNA, full-wave rectified signals of RSNA, as well as background noise signals, were obtained. The noise signal component, which was defined as the signal recorded post mortem, was subtracted from rectified RSNA. To quantify RSNA responses to EPR stimulation, baseline values were calculated by averaging 30 s of data immediately prior to the onset of stimulation and were considered 100% of basal RSNA. Subsequently, changes in RSNA were expressed as a percentage of baseline, and the relative changes in RSNA (ARSNA, %) from baseline were evaluated. Data sets of 1-s averages for MAP, HR, RSNA, and hindlimb tension were analyzed. Baseline values for all variables were determined by evaluating 30 s of recorded data before a muscle contraction. The peak response of each variable was defined as the greatest change from baseline elicited by contraction.

Statistical Analyses

Data were analyzed using unpaired t-tests to identify differences between specific group means. The significance level was set at \( P < 0.05 \). Results are presented as means ± SE.

RESULTS

Morphometric characteristics, biochemical changes, as well as baseline hemodynamics, are presented in Table 1. The heart weight-to-body weight ratio tended to be higher in the 1.2% Pi than the 0.6% Pi diet group, but the difference did not reach statistical significance (2.89 ± 0.10 vs. 2.67 ± 0.04 mg/g, respectively, \( P = 0.051 \)). There were no significant differences in the heart weight-to-tibial length ratio or the lung weight-to-body weight ratio between the two groups. Twenty-four hour urinary excretion of calcium (Ca), sodium (Na), and potassium (K) were not significantly different between the two groups. Twenty-four-hour urinary Pi excretion in the 1.2% Pi group was markedly higher compared with the 0.6% Pi group (77 ± 5 vs. 12 ± 1 mg/day, respectively, \( P < 0.01 \)). There was no significant difference in serum creatinine or creatinine clearance between the two groups (0.35 ± 0.01 vs. 0.37 ± 0.01 mg/dl, respectively, \( P = 0.3 \) and 2.83 ± 0.17 vs. 2.64 ± 0.30 ml/min, respectively, \( P = 0.6 \)). Additionally, there were no

![Fig. 1. Characteristic cardiovascular and sympathetic responses during static muscle contraction in representative Sprague-Dawley (SD) rats fed with 0.6% dietary phosphate (Pi) (left) and 1.2% Pi (right) for 12 wk. The contraction-induced increases in arterial blood pressure (ABP), as well as raw and normalized renal sympathetic nerve activity (RSNA, %) were larger in the SD rat treated with 1.2% Pi compared with 0.6% Pi diet.](http://ajpregu.physiology.org/)
Fig. 2. Summary data showing cardiovascular and sympathetic responses to activation of the exercise pressor reflex (EPR) during static muscle contraction in the 0.6% Pi (n = 13) and 1.2% Pi (n = 13) rats. The time course of changes in mean arterial pressure (MAP), heart rate (HR), renal sympathetic nerve activity (RSNA), and muscle tension are shown in A, D, G, and J. The peak MAP, HR, RSNA, and muscle tension responses to muscle contraction are shown in B, E, H, and K. The integrated changes in MAP, HR, RSNA, and muscle tension presented as area under the curve (AUC) over 30 s are shown in C, F, I, and L. *P < 0.01 compared with 0.6% Pi.
differences in urine volume, food intake, serum Pi, Ca, Na, and K between the two groups (all P values > 0.1).

Intake of a high Pi diet for 3 mo significantly increased tail cuff systolic BP compared with consumption of a normal Pi diet (109 ± 3 vs. 95 ± 2 mmHg, respectively, P < 0.001). Similarly, resting mean BP (120 ± 5 vs. 93 ± 4 mmHg, P < 0.01), as well as HR (355 ± 5 vs. 333 ± 9 beats/min, P < 0.05) under 1% isoflurane anesthesia was significantly higher in the 1.2% Pi group compared with the 0.6% Pi group. After decerebration, however, there were no significant differences in baseline hemodynamics between groups. The baseline SNA-to-noise ratio was also not different between the two groups.

The peak BP, HR, and RSNA responses to activation of the EPR during static muscle contraction were significantly augmented in the high Pi group compared with the control group (44 ± 5 vs. 14 ± 2 mmHg, P < 0.01; 14 ± 2 vs. 4 ± 1 bpm, P < 0.01; 126 ± 25 vs. 42 ± 9%, P < 0.01, respectively, Fig. 1 and Fig. 2, B, E, H, and K). Importantly, muscle tension developed during ventral root stimulation was similar between the two groups (1.3 ± 0.1 vs. 1.4 ± 0.1 kg, respectively, P > 0.1, Figs. 1 and 2). The integrated changes in MAP, HR, RSNA, and tension presented as area under the curve (AUC) over 30 s demonstrated similar results (Fig. 2, C, F, I, and L). Dietary Pi loading also enhanced the BP, HR, and RSNA responses to stimulation of the muscle metaboreflex during capsaicin administration compared with the 0.6% Pi group (50 ± 6 vs. 25 ± 4 mmHg, P < 0.01; 15 ± 2 vs. 3 ± 1 bpm, P < 0.01; 129 ± 25 vs. 58 ± 9%, P < 0.05, respectively; P < 0.05, Figs. 5 and 6). The integrated changes in MAP and HR were significantly higher in the 1.2% Pi compared with the 0.6% Pi group (Fig. 6, C and F; P < 0.01). The AUC of RSNA during capsaicin administration tended to be higher in in the 1.2% Pi compared with the 0.6% Pi group, but the difference did not reach statistical significance (P = 0.086, Fig. 6). The major findings from this investigation are threefold. First, consumption of a high-Pi diet augments cardiovascular and sympathetic responses to EPR stimulation during muscle contraction. Second, high dietary Pi intake potentiates BP, HR, and RSNA responses to both passive muscle stretch and

**DISCUSSION**

The major findings from this investigation are threefold. First, consumption of a high-Pi diet augments cardiovascular and sympathetic responses to EPR stimulation during muscle contraction. Second, high dietary Pi intake potentiates BP, HR, and RSNA responses to both passive muscle stretch and
Fig. 4. Summary data showing cardiovascular and sympathetic responses to activation of the mechanically sensitive component of the EPR in the 0.6% Pi (n = 13) and 1.2% Pi (n = 13) rats. The time course of changes in MAP, HR, RSNA, and muscle tension are shown in A, D, G, and J. The peak MAP, HR, RSNA, and muscle tension responses to muscle stretch are shown in B, E, H, and K. The integrated changes in MAP, HR, RSNA, and muscle tension, which are presented as area under the curve (AUC) over 30 s, are shown in C, F, I, and L. *P < 0.01 compared with 0.6% Pi.
intra-arterial capsaicin administration in the hindlimb, suggesting enhanced mechanoreflex and metaboreflex function. Third, this detrimental effect of a high-Pi diet occurs in the absence of renal failure, a disease condition known to be associated with hypertension and augmented EPR function (35, 36). Collectively, our present study provides the first direct evidence that chronic exposure to a high-Pi diet in otherwise healthy animals induces sympathetic overactivation during EPR stimulation, resembling the phenotype observed in rodent models of non-Pi-induced hypertension (28, 30).

The high Pi diet used in our study had the content that mimics the excess Pi consumed by the general American population as it contained twice the amount of total Pi compared with the control diet (1.2% vs. 0.6% total Pi) and was enriched with inorganic Pi (0.9% vs. 0.3% inorganic Pi). The extreme elevation in 24-h urinary Pi excretion in the high Pi group is reflective of the exceedingly absorbable nature of inorganic Pi. Our study in rodents has revealed a novel action of Pi on the neural control of BP during physical exertion as the high-Pi diet unequivocally potentiated the increase in SNA, BP, and heart rate to muscle contraction compared with the resting condition. The sympathoexcitation induced by the high-Pi diet was mediated by both functional components of the exercise pressor reflex: the muscle mechanoreflex and metaboreflex. Although previous studies indicated that high-salt intake enhanced EPR function in normotensive SD rats (28, 51), our current data indicated a similar property of dietary Pi, which is independent of Na intake, as evidenced by equivalent 24-h urinary Na excretion rates.

The mechanisms underlying Pi-induced enhancements in EPR function remain unknown. Ingestion of an excess amount of inorganic Pi has been shown to promote renal injury in rodents (10, 25). Furthermore, previous studies in patients with chronic kidney disease have demonstrated augmented EPR function, possibly via enhanced mechanoreflex function (35, 36). However, impaired renal function is not likely to explain our study results for two reasons. First, serum creatinine and creatinine clearance in the high-Pi group were identical to the control group. Second, the decline in renal function associated with high dietary Pi was not typically observed in rodents with intact kidneys until total Pi content exceeded 2% (10, 33). A high Pi diet has also been shown to induce cardiomyopathy in mice with normal renal function (12). Previous studies in cardiomyopathic rats and patients with congestive heart failure have demonstrated enhanced EPR activity via selective mechanoreflex sensitization (27, 41, 43). In our study, rats subjected to chronic exposure of a high-Pi diet tended to have increased heart weight-to-body weight ratios, suggesting development of left ventricular hypertrophy. However, the lung weight-to-body weight ratio in the high Pi group was not increased, indicating that congestive heart failure had not developed. Thus, it is unlikely that cardiomyopathy induced by a high-Pi diet is the primary cause of EPR dysfunction.

Aside from indirect effects of Pi on renal or cardiac function, another possibility includes direct stimulatory effects of Pi on the autonomic or preautonomic neurons in the central nervous system. Sodium-phosphate transporters (NaPi-2c) have been identified in many diencephalic regions, including the amygdala and third ventricle (11, 31). Expression of these transporters is altered by dietary Pi intake and concentration of Pi in the cerebrospinal fluid (CSF) (31). Another Pi transporter, PiT2, has also been identified in the choroid plexus of sharks and implicated in the removal of Pi from the CSF (9). Dietary Pi restriction has been shown to reduce Pi levels in the serum and CSF, which was accompanied by increased Pi appetite and Pi-seeking behavior within 48 h (5, 49). In our study, serum Pi
was not altered by the high-Pi diet, although the 24-h urinary Pi excretion was significantly increased. Whether consumption of the high-Pi diet induced an elevated Pi milieu in the brain, causing direct stimulation of brain stem centers involved in the generation of muscle reflex-induced central sympathetic outflow remains to be determined.

In addition to the potential direct effect of Pi on the sympathetic nervous system, consumption of a diet high in phosphate may alter baroreflex sensitivity constituting another mechanism by which EPR function could be augmented. It has been demonstrated that the baroreflex normally acts to restrain EPR-induced increases in BP and RSNA (42). In our study, the 1.2% Pi group displayed both elevated resting heart rate and blood pressure, suggesting impaired baroreflex control of heart rate at rest. Reductions in sensitivity could compromise the buffering capacity of the baroreflex contributing to the EPR overactivity observed. Future studies are needed to assess the effects of high dietary Pi on baroreflex control of blood pressure, heart rate, and SNA during exercise. Exposure to a high-Pi environment may induce alterations in the levels of

Fig. 6. Summary data showing cardiovascular and sympathetic responses to activation of the metabolically sensitive component of the EPR in the 0.6% Pi (n = 13) and 1.2% Pi (n = 13) rats. The time course of changes in MAP, HR, and RSNA are shown in A, D, and G. The peak MAP, HR, RSNA, and tension responses to intrafemoral capsaicin are shown in B, E, and H. The integrated changes in MAP, HR, and RSNA, which are presented as AUC over 30 s, are shown in C, F, and I. *P < 0.05 compared with 0.6% Pi.
many hormones involved in Pi homeostasis, which could affect EPR function. Previous studies demonstrated that acute Pi loading by either enteral or intravenous routes induced release of phosphaturic hormones, including fibroblast growth factor 23 (FGF-23) and parathyroid hormone (PTH) (3, 7, 38, 39). FGF23 is a major hormone released from osteocytes in bone to augment renal phosphate excretion, thereby minimizing phosphate overload (19, 37). This action of FGF23 requires klotho, which functions as a coreceptor for transduction of FGF23 signaling (13). A high-Pi diet has also been shown to reduce soluble klotho levels in the serum of mice (12). Soluble klotho is a multifunctional protein present in biological fluids, including blood, urine, and CSF. Whether a high-Pi diet induces hypertension and sympathetic overactivation via increased expression of FGF23 and/or PTH or downregulation of klotho remains to be determined.

Pi is an essential component of many biologic molecules and is crucial for a large number of cellular functions (21). The recommended daily allowance (RDA) of phosphorus in adults is 700 mg/day, according to the Institute of Medicine (44). Pi derived from vegetables or plants, which contain mainly organic phosphate, is poorly absorbed because it is present in the nonhydrolyzable phytate form (16, 17). Unfortunately, the Western diet is well known to contain enormous amounts of inorganic Pi, which is readily absorbed in the gastrointestinal tract (17). Inorganic Pi-containing food additives were found to be present in more than 40% of top-selling grocery items, including frozen foods, dry food mixes, packaged meat, and soup (22). It is estimated that an average U.S. adult consumes Pi of ~1,200 mg/day, which is almost twice the recommended daily allowance (4). Emerging evidence suggests that excessive phosphate intake can potentially instigate a number of pathological sequelae, including atherosclerosis, myocardial infarction, vascular calcification, and stroke in patients with and without renal disease (4, 18, 26). Adding to this list, our study demonstrated that dietary Pi excess detrimentally affects the sympathetic regulation of BP during exercise by potentiating the function of both the muscle mechanoreflex and metaboreflex, the two major components of the EPR and, in doing so, may contribute to the pathogenesis of Pi-induced hypertension. Just as important, numerous epidemiological studies have demonstrated that exercise BP predicts subsequent development of stroke, myocardial infarction, and death (20, 23, 24, 47), independent of resting BP.

Our study is limited by several factors, including a lack of molecular insight into mechanisms linking high dietary Pi to the pathogenesis of EPR dysfunction. Moreover, measurement of BP yielded lower levels of conscious BP than those obtained during isoflurane anesthesia, which was unexpected. However, the tail cuff measurement was limited to a single time point, which may not reflect fluctuations in BP over the longer time period analyzed under anesthesia. Despite the limitations of this technique, both tail cuff BP and BP under anesthesia were higher in the 1.2% Pi group compared with 0.6% Pi group. Thus, our study demonstrated a consistent BP-raising effect of dietary Pi loading in otherwise normal rodents. Our study may provide an explanation for the elevation in SNA and augmented EPR function in patients with chronic kidney disease (35, 36), who are prone to develop Pi retention and hypertension. If our findings are confirmed in humans, our study results would call for a revision in the food-labeling process to include inorganic phosphate. As with Na, such a revision would not only allow the American public to monitor their phosphate intake but also serve as a warning that their health could be compromised by excess consumption of Pi.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES

11. Hisano S, Haga H, Li Z, Tatsumi S, Miyamoto KI, Takeda E, Fukui Y. Immunohistochemical and RT-PCR detection of Na+-dependent inor-


