Evidence for induction of a phosphate appetite in juvenile rats

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Sweeny, Joseph M., H. Edward Seibert, Craig Woda, Jay Schulkin, Aviad Haramati, and Susan E. Mulroney. Evidence for induction of a phosphate appetite in juvenile rats. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1358-R1365, 1998.—This study examined whether dietary phosphate (Pi) restriction stimulates an appetite for Pi in the juvenile rat, which normally has a high metabolic Pi demand for growth. Juvenile Wistar rats were placed in individual cages with unrestricted access to tap water and a low (LPD, 0.02% Pi) or normal Pi diet (NPD, 0.6% Pi) for 7 days. On day 8, both groups of rats were given unlimited access to a solution of 0.3 M potassium phosphate water (PiH2O) for 8 additional days. Rats fed LPD consumed 70–100% more PiH2O than those rats fed NPD (Pi < 0.001). The increase in PiH2O intake resulted in a marked rise in the growth rate of rats fed LPD during days 8–15. A similar Pi intake was inducible after only 2 days of LPD and was associated with significant reductions in both plasma and cerebrospinal fluid (CSF) Pi levels; these levels remained low throughout Pi restriction, despite a significant PiH2O intake. Furthermore, the renal adaptation to enhance Pi reabsorption (TmPi) during Pi deprivation remained elevated despite enhanced PiH2O intake. Replenishment with a high-Pi diet rapidly quenched the PiH2O appetite and was associated with restoration of both plasma and CSF Pi levels. These findings suggest that an appetite for Pi can be induced in juvenile rats, perhaps through lowered plasma and CSF Pi levels. This behavioral response may serve as an additional mechanism to maintain an adequate supply of Pi, necessary for growth and development of the animal.

HISTORICAL RECORDS dating back to 1785 recount observations made by travelers to South Africa concerning a peculiar behavior in cattle. These records describe cattle as eagerly searching for and gnawing bones after feeding on what the travelers called “harsh grass” (20). Because of the sickness (and subsequent economic losses) that usually developed in the cattle after the ingestion of decaying skeletal debris, initial investigations were initiated, and this behavior was linked to a mineral deficiency in the cattle.

A similar observation was made in 1925 when Henry Green reported what he termed to be a “perverted appetite” in cattle (16). Up to 90% of the cattle grazing on certain phosphate-deficient soils chewed on skeletal bones (a naturally occurring source of Pi). Green postulated that such behavior was due to a phosphate deficiency, but it was not until later that laboratory experiments performed by Denton et al. (9) confirmed that this osteophagic behavior in cattle was indeed brought on by a phosphate deficiency. By experimentally inducing a chronic (2 yr) Pi deficiency through a parotid fistula and a low-phosphate diet, Blair-West et al. (2) reported bone to be licked, picked up with the mouth, and chewed on much more avidly in the cattle deprived of phosphate compared with replete controls. Furthermore, in young heifers, Pi deficiency was also associated with growth retardation, and the appetitive behavior could be suppressed by raising the plasma phosphate concentration through a rapid intravenous infusion of a buffered Pi solution (6). These observations provide evidence suggesting that the Pi appetite in these cattle is linked to a phosphate deficiency. However, whether this phenomenon occurs in other mammals or is species dependent (rat vs. cattle) and related to an herbivorous or omnivorous behavior remains unknown.

The classic mineral appetite is that displayed for sodium (7, 26, 36). Given the important role sodium has in maintaining extracellular fluid volume, it is not surprising that a behavioral adaptation emerged to help maintain fluid homeostasis during times of sodium depletion (10, 12). Although other mineral deficiencies (e.g., potassium, iron, and calcium) result in sodium ingestion (28), Pi deficiency does not (35). This may suggest that the motivation to obtain Pi in the rat may be specific for Pi, and in fact be an intrinsic response (2).

Pi is essential for growth and development in the immature animal as well as used in a myriad of metabolic processes in the adult animal. Physiological adaptations exist to ensure that an adequate supply of Pi is made available to the growing and adult animal. For example, urinary losses of Pi are limited in young rats, related in part to an enhanced ability to transport Pi. Indeed, the normal tubular capacity of the kidney to reabsorb Pi is enhanced in immature rats compared with adult rats (3, 18) and appears to be related to the increased demand for Pi during growth (32). Furthermore, during dietary Pi restriction, both the adult and juvenile animal significantly increase their maximal renal capacity to reabsorb Pi, enhancing PiH2O intake. Replenishment with a high-Pi diet rapidly quenched the PiH2O appetite and was associated with restoration of both plasma and CSF Pi levels. These findings suggest that an appetite for Pi can be induced in juvenile rats, perhaps through lowered plasma and CSF Pi levels. This behavioral response may serve as an additional mechanism to maintain an adequate supply of Pi, necessary for growth and development of the animal.
whether a Pi appetite can be induced in juvenile rats by feeding them a low-P, diet and measuring their potassium phosphate water (P,H2O) intake; 2) to determine whether an appetite for Pi can be stimulated rapidly by 2 days of Pi restriction; 3) to examine whether this appetite is specific for Pi, and not another mineral involved in the growth and development of the young animal (e.g., calcium); and 4) to examine the effects of Pi, deficiency on growth, plasma, and CSF Pi, levels and renal Pi, reabsorption.

METHODS

Animals, diets, and fluids. All experiments were performed using male, juvenile Wistar rats (Harlan, Indianapolis, IN) ranging from 23 to 25 days of age at the onset of testing. After arrival, the rats were initially fed standard Purina Rodent Laboratory Chow pellets (no. 5001) while they adapted to laboratory conditions (2 days). Each rat was individually housed in an 18 × 12 × 10 cm hard plastic cage with a 2-in. bedding of wood chips and fitted with a stainless steel cage top. Animals were housed in a 12:12-h light-dark cycle with lights on from 0700 to 1900. Unless otherwise mentioned, for each study animals were divided into an experimental group designed to receive a low-P diet (LPD, 0.02% Pi), consisting of a basic low-P, rodent diet (Research Diets 93082402, New Brunswick, NJ) supplemented with 9.7 g/kg NaCl and 14.76 g/kg KCl, and a control group that was fed a normal P, diet (NPD, 0.6% Pi), which was the basic LPD supplemented with 3.02 g/kg KH2PO4, 15.46 g/kg K2HPO4, 2.28 g/kg NaH2PO4, and 10.48 g/kg Na2HPO4 salt additives.

The rodent diet and additives were powdered, provided in tall stainless steel bowls to avoid spillage, and placed on the floor in the right front corner of each cage. Dependent on the experiment (short- or long-term Pi deprivation), rats were given access to a 0.3 M solution of KH2PO4 (P,H2O) at physiological pH that was slightly sour yet palatable to human taste. In the experiment to test whether this appetite was specific for Pi, rats fed NPD and LPD were given access to both P,H2O and an equimolar (0.3 M) calcium carbonate (CaH2O) solution. The 0.3 M CaCO3 solution was at physiological pH and had a bitter taste to humans. The concentrations of these solutions were selected in an attempt to provide a solution that would be somewhat aversive to rats.

Throughout the experiments, rats had continuous access to 250 ml of tap water, which was provided in inverted plastic bottles (500 ml, Girton model 16–38). When given, the P,H2O and CaH2O were provided in inverted 100-ml glass bottles. The spouts dispensing the water, P,H2O, and CaH2O were placed at identical levels in the cage (~10 cm from floor). In cases in which rats were required to make a three-way choice among water, P,H2O, or CaH2O, the drinking spouts were placed 7–10 cm apart, and their relative positions were maintained daily.

In each experiment, daily body weight gain and food intake was monitored, and water and salt solution consumption was measured using a 100-ml graduated cylinder. Measurements were obtained at the same time each day. After each measurement, both food and fluids were replenished. The bottles dispensing the P,H2O and CaH2O were cleaned and refilled with fresh fluids every 2 days to avoid precipitate accumulation.

At the end of each experiment, the plasma Pi, levels in both Pi-deprived and -replete controls were determined. Rats were anesthetized intraperitoneally with pentobarbital sodium (100 mg/kg body wt). A 1.5-ml blood sample was collected directly from the heart of each rat in heparinized tubes and centrifuged (10,000 rpm for 5 min), and plasma was extracted and frozen at −70°C. Plasma Pi, concentrations were measured by the phosphomolybdate method of Chen et al. (4).

The Pi, intake was determined as the Pi in the food and P,H2O ingested. The concentration of Pi, in LPD was 0.007 mmol Pi/g food and was multiplied by the daily food intake. The concentration of P,H2O was 0.3 M (300 µmol/ml) and was multiplied by the volume ingested.

Concentration of Pi, in plasma and cerebrospinal fluid. To determine the effects of Pi restriction on plasma Pi, levels, juvenile Wistar rats were fed a LPD and were killed after days 0 (n = 3), 1 (n = 3), 2 (n = 3), and 7 (n = 3) of Pi, restriction. Blood samples were obtained on these days and analyzed for plasma Pi,. These values were compared with plasma Pi, from animals fed LPD and P,H2O. To collect cerebrospinal fluid (CSF), 23-gauge stainless steel guide cannulas were implanted stereotaxically into the third ventricle, as previously reported (24), in rats fed either LPD (n = 4) or NPD (n = 4) for 3 days. Injectors were placed in the guide cannulas, and CSF was drawn into PE-10 tubing. The tubing was sealed at both ends by flame and stored at 4°C until microanalysis. Analysis was performed by using a flow-through both microcolorimeter and a modified phosphomolybdate method of Chen et al. (4). CSF samples (100 nl) or Pi, standards (50 nl) were transferred to separate tubing containing 2 µl of reagent (10% ascorbic acid, 10%8MH2SO4, and 10% 2.5% ammonium molybdate(VI) in distilled water). The samples were sealed, mixed, and incubated in a 37°C water bath for 90 min. After incubation, the samples were injected into the spectrophotometer port, and the absorbance was read as a change in voltage. All samples were run in duplicate in two separate assays, and Pi, concentration was determined against the standard curve.

Effect of 7-day Pi, restriction on Pi, appetite. To assess whether a Pi, appetite could be stimulated in juvenile rats, animals were pair fed either NPD (n = 11) or NPD (n = 11): the NPD rats were fed only the average amount of food consumed by the LPD rats the previous day. On day 7, both LPD and NPD rats were given free access to P,H2O for the remaining 3 days of the experiment. Body weight gain and food and water intakes were monitored daily throughout the experiment. Plasma Pi, was determined at the end of the experiment as previously described.

Effect of 2-day Pi, restriction on Pi, appetite. To examine whether an appetite for Pi, could be rapidly induced after just 2 days of Pi, deprivation, juvenile rats were fed LPD (n = 6) and NPD (n = 6) for 9 days. After 2 days, rats fed both NPD and LPD received unlimited access to the P,H2O solution for the remaining 7 days. Body weight gain and food and water intakes were monitored daily. Plasma Pi, was determined at the end of the experiment.

Effect of 2-day Pi, deprivation on renal tubular Pi, reabsorption. To monitor the renal effects of dietary Pi, deprivation, a separate group of rats was placed on a NPD or LPD, and after 2 days, acute renal clearances experiments were performed (23) to assess the maximal capacity for tubular Pi, reabsorption (Tm,PI) during changes in dietary phosphate intake. Briefly, animals were fed their respective diets (NPD, n = 3; LPD, n = 5). Another group of animals fed LPD were given access to P,H2O, and Tm,PI was assessed after 5 days (n = 5). On the day of the acute experiment, the rats were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg, Promonta, Hamburg, Germany) and placed on a heated table. Body temperature was monitored via a rectal probe and maintained at 37 ± 0.5°C with a heated table and lamp.
tracheostomy was performed, and a tube was placed in the airway so the animals could breathe spontaneously. Catheters were placed in the jugular vein for infusions, carotid artery for blood pressure measurements and blood sampling, and the bladder for urine collection. Animals were then acutely thyroparathyroidectomized by heat cautery to remove the influence of endogenous parathyroid hormone. A 2% inulin solution was infused at 2% body weight/h throughout the experiment. After a 2-h recovery period, a 20-min baseline urine clearance was taken. To determine the \( T_{\text{mPi}} \), increasing concentrations of \( P_i \) (calculated to deliver 3–9 \( \mu \text{mol/min} \)) were added to the inulin solution and infused to increase the filtered load of \( P_i \). Urine collections were made every 20 min for 2–3 h. Blood was sampled midway through each clearance period, and after the experiment, the animals were killed. Inulin concentrations in urine and plasma samples were determined by the anthrone method (15), and the glomerular filtration rate (GFR) was equated with the clearance of inulin.

The \( T_{\text{mPi}} \) was calculated as the mean of the highest individual values of reabsorbed \( P_i \) per milliliter GFR in each group. Specificity of \( P_i \) appetite. Because both \( P_i \) and calcium are essential components of developing bone (as hydroxyapatite), a preliminary assessment of whether short-term (2-day) \( P_i \) deprivation in juvenile rats induces an appetite specific for \( P_i \), and not calcium was performed. Juvenile rats were fed LPD (\( n = 5 \)) and NPD (\( n = 5 \)) over 10 days. After 2 days, both LPD and NPD were given equal access to separate but equimolar (0.3 M) \( P_i \)-H\(_2\)O and CaH\(_2\)O solutions as well as tap water for the remaining 8 days. Body weight gain and fluid and food intakes were monitored.

\( P_i \) appetite and plasma and CSF \( P_i \) concentrations after \( P_i \) replacement. The working hypothesis is that, during \( P_i \) deprivation, reductions in plasma and CSF \( P_i \) levels stimulate and maintain the behavioral response in the juvenile animal. Furthermore, it is proposed that if plasma and/or CSF \( P_i \) levels were restored to normal, the behavioral response to ingest additional \( P_i \) would be turned off. To test this notion, juvenile rats were fed NPD (\( n = 3 \)) or LPD (\( n = 4 \)) for 7 days. Body weights and food and water intake were monitored throughout the experimental period. After 2 days on the respective diets, animals were given free access to 0.3 M \( P_i \)-H\(_2\)O for 5 additional days, and \( P_i \) appetite was monitored. On the 7th day of the experiment (after 5 days on \( P_i \)-H\(_2\)O), all animals were placed on a high-\( P_i \)-diet (HPD) for 3 days while \( P_i \)-H\(_2\)O was still available. On the 10th day, animals were anesthetized, and canulas were placed in the third ventricle of the brain to obtain a CSF sample. Blood was also obtained at the end of the experiment for determination of plasma \( P_i \) levels.

Statistical analysis. Comparisons within and between the groups were obtained by using paired and unpaired Student’s t-tests, respectively. The rate of growth over the experimental periods was equated with the slope (determined by linear regression) of the individual growth curves. Means \( \pm SE \) were determined for the slopes of each pre- and post-\( P_i \)-H\(_2\)O group. Significance was designated as \( P < 0.05 \), and all results are expressed as means \( \pm SE \).

RESULTS

Effect of 7-day \( P_i \) restriction on \( P_i \) appetite. Rats fed LPD demonstrated a significantly reduced growth rate during the first 7 days of the experiment compared with animals fed NPD (Fig. 1). From days 0 to 7, the average daily gain in body weight in NPD rats was 4.5 \( \pm 0.4 \) g/day compared with 1.5 \( \pm 0.4 \) g/day in LPD-fed rats (\( P < 0.05 \)). Once the \( P_i \)-H\(_2\)O was provided to both NPD and LPD rats after day 7, the LPD rats showed an increase in daily body weight gain for the remaining 8 days that matched that observed in controls (6.0 \( \pm 0.6 \) vs. 6.1 \( \pm 0.5 \) g/day over 8 days in NPD, \( P < 0.05 \)). Relative slopes of the growth curves were 4.23 \( \pm 1.17 \) and 1.24 \( \pm 1.27 \) for rats fed NPD and LPD, respectively, during days 0–7 (\( P < 0.01 \)). The slope for days 8–15 (6.21 \( \pm 0.92 \)) reflects the increased growth rate of rats fed LPD plus \( P_i \)-H\(_2\)O (\( P < 0.001 \) vs. LPD days 0–7). This elevated rate of growth was not different from that observed in rats fed NPD over days 8–15 (6.36 \( \pm 0.67 \)). Over days 8–15, rats fed LPD plus \( P_i \)-H\(_2\)O also consumed more food (Fig. 2, top; 10 \( \pm 0.2 \) over days 0–7 vs. \( 14 \pm 0.3 \) g/day over days 8–15, \( P < 0.05 \)). This may also explain the increased daily body weight gain in the pair-fed rats fed NPD over days 8–15. Ingestion of \( P_i \)-H\(_2\)O was significantly greater in the animals fed LPD compared with the NPD controls over days 8–15 (3.0 \( \pm 0.2 \) vs. 1.8 \( \pm 0.1 \) ml/day; Fig. 3). This \( P_i \)-H\(_2\)O intake corresponds to ingestion of \(-0.9 \text{ mmol} P_i \text{/day in the rats fed LPD. This was less than one-half of the normal amount of} P_i \text{ ingested by the animals eating NPD (}-2.1 \text{ mmol/day). Interestingly, rats fed NPD also exhibited a growth rate increase during the period that} P_i \text{-H}\(_2\)O was provided (days 0–7, 4.5 \( \pm 0.4 \) g/day, compared with days 8–15, 6.1 \( \pm 0.5 \) g/day), but this increase may be a result of the pair-fed design of the experiment and an increase in food ingestion in LPD-fed rats. Similarly, when \( P_i \)-H\(_2\)O was provided at day 7, water consumption increased in the LPD-fed rats (Fig. 2, bottom).

Effect of 2-day \( P_i \) restriction on \( P_i \) appetite. To determine whether the induction of a \( P_i \) appetite was a rapid process, a separate group of animals was given access to \( P_i \)-H\(_2\)O after only 2 days of \( P_i \) deprivation. At this time point, the renal adaptation to \( P_i \) restriction was already evident, as seen by a significant \( T_{\text{mPi}} \) elevation in rats fed LPD compared with those rats fed NPD (8.7 \( \pm 0.6 \) vs. 5.6 \( \pm 0.1 \) mmol/ml GFR in NPD controls, \( P < 0.001 \)). These \( P_i \)-restricted animals also exhibited an increase in \( P_i \)-H\(_2\)O consumption (3.2 \( \pm 0.2 \) ml/day) compared...
with the controls (1.8 ± 0.2 ml/day), which was similar to that observed after 7 days of P i restriction (Fig. 3). The reduction in daily body weight gain observed in rats fed LPD (0.1 ± 0.4 g/day; slope of 0.33 ± 0.89 vs. 6.30 ± 1.39 in rats fed NPD, P < 0.0001) was significantly increased during P i ingestion (average daily gain in body weight during days 3–9 of 4.2 ± 0.5 g/day; slope of 3.71 ± 2.17 vs. 5.28 ± 0.34 in rats fed NPD, P = 0.14; P < 0.001 vs. 2-day LPD). Animals fed NPD during this period maintained a constant, rapid growth rate throughout the experimental period. Interestingly, the animals fed LPD during this period continued to exhibit the P i appetite during the entire period: the maintenance of the P i appetite was also associated with continued low plasma P i levels (1.9 ± 0.1 vs. 3.2 ± 0.1 mM in NPD controls, P < 0.01) as well as elevated T mP i (8.1 ± 0.3 µmol/ml GFR, P < 0.001 vs. NPD controls, but NS to LPD controls; Fig. 4). This indicated that both the behavioral and physiological adaptations were still stimulated, despite the increased supply of P i, but suggests that the ingestion of P iH2O was not adequate to restore the system to normal.

P iH2O ingested by rats fed LPD and NPD after long-term P i restriction was nearly identical to short-term P i-restricted rats, indicating that the longer P i deprivation had no additional effect on the P i appetite. At either time point (2- or 7-day P i deprivation), plasma P i levels were still significantly lower in rats fed LPD and P iH2O compared with those fed NPD and P iH2O. A period of P i deprivation in excess of 7 days may, however, yield additional information.

Specificity of P i appetite. Juvenile rats fed NPD and LPD for 2 days exhibited a preferential intake of P iH2O when given a choice between equimolar P iH2O and CaH2O solutions. There was no significant difference in CaH2O ingestion between animals fed NPD or LPD (0.2 ± 0.4 vs. 0.5 ± 0.5 ml, respectively). However, during the same period, rats fed LPD ingested an average 1.6 ± 0.3 ml/day of P iH2O, whereas rats fed NPD ingested 0.7 ± 0.1 ml/day (P < 0.002). In addition, the increased consumption of P iH2O in LPD rats was accompanied by an increased average growth rate of 3.4 ± 0.5 g/day during days 3–10 (P < 0.007 vs. 0.4 ± 0.6 g/day during initial 2 days of LPD). The amount of P iH2O ingested was consistent over the entire period,
indicating that the Pi appetite was still in effect. Again, the maintenance of the Pi appetite was associated with significantly lower plasma Pi levels in LPD-fed rats receiving P,H,O (1.78 ± 0.15 mM) compared with those rats fed NPD also receiving P,H,O (3.19 ± 0.15 mM, P < 0.0001).

Plasma and CSF Pi concentrations during Pi restriction. Because the Pi appetite was evident early (after 2 days of Pi deprivation), we assessed the time course for changes in plasma Pi concentrations. Plasma Pi levels after 0, 1, 2, 3, and 7 days of Pi restriction are shown in Fig. 5. By day 2 of Pi restriction, the plasma Pi levels were significantly decreased from control levels (1.79 ± 0.03 vs. 2.79 ± 0.16 mM in controls, P < 0.003). This reduction in plasma Pi was not different after 7 days of Pi restriction. Furthermore, CSF Pi levels in rats fed LPD for 3 days were also significantly reduced compared with controls (0.20 ± 0.01 vs. 0.47 ± 0.01 mM in rats fed NPD, P < 0.05).

Pi appetite, plasma and CSF Pi concentrations after Pi replenishment. To assess whether plasma and/or CSF Pi levels are involved in the maintenance of the Pi appetite, a high concentration of Pi was replenished in the diets of Pi deficient juvenile rats. Figure 6 (left) summarizes data from the previous experiments. As previously observed, ingestion of Pi,H,O was stimulated in juvenile rats fed LPD after only 2 days of dietary Pi deprivation (1.6 ± 0.2 vs. 0.8 ± 0.1 ml P,H,O/day in NPD controls, P < 0.001; Fig. 6, bottom left). At this time, plasma and CSF Pi levels are still significantly reduced despite 5 days of ingestion of Pi,H,O (Fig. 6, top and middle left, respectively).

In the additional set of animals, the ingestion of Pi,H,O was again associated with a significant (P < 0.04) increase in growth rate in the rats fed LPD (3.9 ± 0.7 g/day) compared with their growth rate over the two days of LPD only (1.6 ± 0.6 g/day). The increased growth rate in rats fed LPD and ingesting Pi,H,O was not due to alterations in food intake (13.2 ± 0.5 g food/day over 1st 2 days vs. 14.0 ± 0.5 g food/day over 5 days on Pi,H,O, not significant). Replenishment of Pi in the diet (as HPD) did not change food intake in the animals initially fed LPD (11.9 ± 1.2 g/day of HPD over 3 days vs. 14.0 ± 0.5 g/day of LPD); however, there was a further, significant increase in body weight gain to 8.5 ± 1.0 g/day compared with weight gain while on Pi,H,O alone. Replenishment of Pi in the diet of animals initially fed LPD plus Pi,H,O suppressed the ingestion of Pi,H,O within 1 day to the level observed in rats fed NPD plus Pi,H,O (0.8 ± 0.1 vs. 0.8 ± 0.1 ml P,H,O/day in NPD rats, not significant; Fig. 6, bottom right).
Feeding HPD to the animals initially fed NPD had no effect on P\textsubscript{H2O} ingestion (0.8 ± 0.1 vs. 0.8 ± 0.1 ml P\textsubscript{H2O}/day in NPD and HPD animals, respectively). Most importantly, the quenching of the P\textsubscript{i} appetite during dietary P\textsubscript{i} replenishment was associated with a restoration of both plasma and CSF P\textsubscript{i} levels (Fig. 6, top and middle right, respectively).

**DISCUSSION**

The present study indicates that when juvenile rats are deprived of phosphate in their diet, plasma and CSF P\textsubscript{i} levels decrease rapidly, the kidney increases P\textsubscript{i} reabsorption, and the consumption of P\textsubscript{H2O} is significantly increased. Interestingly, the ingestive response was not different between acute (2-day) and longer (7-day) P\textsubscript{i} restriction. The P\textsubscript{i} deficiency induced in this study resulted in the preferential ingestion of P\textsubscript{H2O} rather than a solution of CaH\textsubscript{2}O (2 minerals necessary for growth of young animals). Moreover, the reduction in plasma and CSF P\textsubscript{i} levels may be part of the mechanisms by which the behavioral response is elicited, since restoration of plasma and CSF P\textsubscript{i} levels was associated with quenching of the P\textsubscript{i} appetite. Thus stimulation of an appetite for P\textsubscript{i} may provide a behavioral mechanism, in addition to the intrinsic increase in renal P\textsubscript{i} transport, by which the young animal can increase P\textsubscript{i} supply during periods of phosphate depletion.

It is well known that P\textsubscript{i} is crucial to the proper growth, metabolic processes, and general well being of animals. Reducing the supply of P\textsubscript{i} in juvenile animals results in an immediate attenuation in somatic growth (21, 30). This phenomenon was readily observed in the present study, and consequently a positive change in the growth rate was used as the criterion for the efficacy of oral P\textsubscript{i} ingestion. Indeed, after both 2 and 7 days of phosphate restriction, the stimulation of P\textsubscript{i} ingestion resulted in significant increases in whole body weight gain. This positive correlation highlights the potential interrelationships between the behavioral and physiological responses to P\textsubscript{i} deprivation and growth in the juvenile animal.

Because our studies were focused on the rapidly growing animal, which already has a high demand for P\textsubscript{i}, it is unclear whether a comparable appetite for P\textsubscript{i} can be stimulated in the adult rat. P\textsubscript{i} is also of great importance to metabolic functions and bone remodeling in the adult animal (32), and we hypothesize that adult animals would exhibit a similar appetite to increase P\textsubscript{i} supply, which could be exaggerated in times of high-P\textsubscript{i} demand. The P\textsubscript{i}-seeking behavior observed by previous investigators in cattle grazing on phosphate-poor vegetation support this notion (2, 6, 16). Typically, cows also have a high demand for P\textsubscript{i} to maintain an adequate supply of the mineral for pregnancy and milk production. It is important to note, however, that herbivorous and omnivorous diets, as well as age and developmental status, may affect the degree of appetite induced for different minerals.

One of the effects of reducing dietary P\textsubscript{i} is a decrease in plasma P\textsubscript{i} concentration. In previous studies on cattle, the bone-gnawing behavior increased over months and was associated with a fall (over weeks) in plasma P\textsubscript{i}, and in these studies, P\textsubscript{i} concentrations in CSF were not shown to decrease (2). Denton et al. (9) and Blair-West et al. (2) have proposed that plasma P\textsubscript{i} may regulate the P\textsubscript{i} appetite in cattle. In the present study, there was a rapid fall in plasma P\textsubscript{i} levels in juvenile rats, which was maximal after only 2 days and remained at the same low level through 7 days of P\textsubscript{i} restriction. Furthermore, CSF P\textsubscript{i} levels were also significantly reduced after only 3 days of P\textsubscript{i} restriction, and the reduction most likely occurs earlier. This rapid reduction in peripheral and central phosphate concentrations, which coincided with induction of the P\textsubscript{i} appetite, supports the concept that plasma and CSF P\textsubscript{i} levels, directly or indirectly, are part of the mechanism to stimulate a central behavioral response to increase P\textsubscript{i} ingestion. Although the increase in P\textsubscript{H2O} ingestion was significant enough to increase body weight gain in the animals, it evidently was not enough to restore plasma and CSF P\textsubscript{i} concentrations and potentially turn off the appetite and renal adaptations. This notion is supported by the findings that a greater replenishment of P\textsubscript{i} through HPD quenched the appetite and normalized plasma and CSF P\textsubscript{i} levels. Further studies will help determine the temporal sequence of events contributing to the quenching of the appetite.

Although 7 days of P\textsubscript{i} deprivation has been shown to elicit renal adaptations (18), the 1-wk time period may not have been sufficient to reduce plasma P\textsubscript{i} concentrations enough to stimulate additional P\textsubscript{H2O} consumption. Still, our findings argue against this notion, since although the P\textsubscript{H2O} ingested was enough to significantly increase the growth rate, the plasma P\textsubscript{i} level was not normalized, and both the renal and behavioral adaptations were still active. In light of these findings, we hypothesize that the P\textsubscript{H2O} was aversive enough to restrict more avid ingestion (and hence animals fed LPD only received ~50% of phosphate ingested by animals fed NPD), the plasma and CSF P\textsubscript{i} levels could not return to normal with the daily intake of P\textsubscript{H2O}. In this scenario, the additional P\textsubscript{i} consumption (via P\textsubscript{H2O}) may have been available immediately for metabolic processes rather than remaining in the plasma pool. This could account for the lack of normalization of the plasma and CSF P\textsubscript{i} levels and subsequent maintenance of the behavioral and physiological adaptations.

A potentially important finding was that the P\textsubscript{i} appetite in the juvenile rat induced a preferential intake of P\textsubscript{H2O} over CaH\textsubscript{2}O in this preliminary study. Whether this highlights the relative importance of P\textsubscript{i} in particular to the metabolic and growth requirements of the animal is unknown, and further study is required to fully assess the specificity of the behavioral response. Furthermore, we do not know to what extent the P\textsubscript{i} ingestion is or is not similar to the well-characterized sodium appetite. First reported by Richter (26), the
specific appetite for sodium that emerges when extracellular fluid and sodium are depleted is innate, linked to both a specific gustatory transduction mechanism involving cranial nerves (14a, 27, 31) and excitatory and inhibitory humoral signals that act in the central nervous system (8, 11, 13, 14, 29, 34). As with Pi deficiency, sodium restriction in the young severely attenuates somatic growth, highlighting its importance developmentally.

In conclusion, our initial results support the notion that there may be behavioral as well as the previously reported intrinsic renal mechanisms contributing to the regulation of Pi homeostasis in the rat. The appetite for Pi may be particularly conspicuous during development and, by analogy, during reproduction, when the demands for Pi are high. Moreover, it is conceivable that reductions in plasma and CSF Pi levels affect transport in critical regions of the brain that regulate the Pi appetite and may provide the mechanism by which the behavioral response is mediated.

Perspectives

The notion that phosphate depletion can elicit a rapid behavioral response in juvenile animals, in addition to the renal and hormonal physiological responses, provides a potentially intriguing aspect to this mineral appetite. Although future studies will focus on the specificity of this ingestive behavior and whether it is innate or learned, the findings that the Pi appetite may aid growth in the young is an intriguing aspect of this work and also should be investigated. Hence, the earlier reports of phosphate-seeking behavior in cattle and our present findings of a rapid-onset phosphate appetite in juvenile rats help extend the information regarding mineral appetites and provide another pathway by which phosphate homeostasis may be maintained in the young.

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REFERENCES