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mRNA Expression of *Phex* in Mice and Rats

The Effect of Low Phosphate Diet

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Phex **is the gene whose mutation is the cause of X-linked hypophosphatemia in humans and mice. The organs expressing** ***Phex*** **in normal animals, and their possible sensitivity to stimulation by low phosphate diets, are unknown. In this study,** ***Phex*** **expression was measured in 6-wk-old normal B6C3H male and female mice and in 135 g Sprague-Dawley rats fed a normal phosphate diet or a low phosphate diet with deionized water** ***ad libitum*** **for 7 d. The animals were then anesthetized, and a variety of organs were collected and frozen in liquid nitrogen.** ***Phex*** **mRNA expression was measured in each organ by reverse transcription–polymerase chain reaction (RT-PCR) with primers specific for both** ***Phex*** **and glyceraldehyde-3-phosphate dehydrogenase (G3PDH). Southern blots were prepared, hybridized with ^{32}P-labeled internal oligonucleotides, and quantified with a phosphor imager. The** ***Phex*****/G3PDH ratio was computed, and the data were compiled as the mean ± SEM. In these growing animals, the highest** ***Phex*** **expression levels were found in the gonads, brain, and lung. In contrast,** ***Phex*** **expression in calvaria and femur was markedly less. Two significant changes were found in animals that were fed a low phosphate diet. Spleen showed a significant decrease in** ***Phex*** **mRNA levels on low phosphate diet (60 ± 10% of normal P diet,** ***n*** **= 12/group,** ***p*** **= 0.002). The pituitary gland showed a significant increase in** ***Phex*** **expression with low phosphate diet (851 ± 127% of G3PDH) over normal P diet (569 ± 78%,** ***n*** **= 24 – 25/group,** ***p*** **= 0.03). No significant change was found in femur, calvaria, or a variety of soft tissues. In summary,** ***Phex*** **mRNA was found in most tissues examined. Expression levels varied by two orders of magnitude from highest to lowest with more in gonads, brain, and lung and with less in bone. Increased** ***Phex*** **mRNA was found in the pituitary gland of animals that were fed a low phosphate diet.**

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Introduction

X-linked hypophosphatemia is a genetic disease, which occurs in both humans *(1)* and mice *(2,3)*. In mice, there are two mutations, *Hyp (2)* and *Gy (3)*, which result in abnormalities resembling human X-linked hypophosphatemia. This disease is characterized by reduced renal retention of phosphate *(1)*, low plasma phosphate *(4)*, a failure of 1,25-dihydroxyvitamin D_3 to increase *(5,6)*, and rachitic and osteomalacic bone disease *(7)*.

The mutated gene in this disease has been identified and sequenced. Analysis of several human families with X-linked hypophosphatemia *(8–10)*, as well as the *Hyp (11)* and *Gy (11,12)* mice, has revealed mutations of a novel gene known as *Phex (8)*. This gene was originally known as *Pex (13,14)*. This gene belongs to a family of membrane-bound endopeptidases whose other members activate or inactivate humoral and cytokine agents *(15)*. The normal roles of this gene in phosphate homeostasis and skeletal mineralization are unknown. This has been recently reviewed by Drezner *(16)*.

Physiological data suggest that X-linked hypophosphatemia has a humoral origin. Parabiosis indicates that the *Hyp* mice produce a positive phosphaturic factor *(17,18)*. This factor can cross a parabiotic union into an attached normal mouse and significantly lower the plasma phosphate by reducing the renal retention of phosphate *(17)*. In addition, renal transplantation has been done between normal and *Hyp* mice *(19)*. *Hyp* kidneys placed in normal mice retain phosphate, while normal kidneys placed in *Hyp* mice have reduced phosphate retention *(19)*. These studies suggest that the low phosphate reabsorption by the kidney in X-linked hypophosphatemia is mediated by a humoral factor and is not an endogenous consequence of the mutation *(19)*.

These findings have led to the hypothesis that a hormone exists for the regulation of phosphate metabolism. *Phex* appears to play a key role in this system, either by activating a phosphate-conserving hormone or inactivating a phosphate-wasting hormone. While attempts are being made to

identify this active factor *(20)*, little is known about either the physiological role(s) of the *Phex* protein or about the organ that secretes the phosphate-regulating hormone.

To try to address these issues, we have sought basic data on the expression of *Phex* in the tissues of normal mice and rats. Mice were used for most organs, and rats were used for tissues too small to study in mice. If *Phex* had had a limited expression domain, this would have suggested a relatively small number of organs to examine for the source of the secretion of a phosphate-regulating hormone. Instead, we have found widespread expression of this gene in many organs and tissues. Therefore, we adopted another strategy to identify possible sources of the phosphate-regulating hormone. We examined these *Phex*-expressing organs for responsiveness to a low phosphate diet. We reasoned that if the hormone caused phosphate retention, a low phosphate diet would stimulate its secretion in order to elevate the renal retention of phosphate and to activate the formation of 1,25-dihydroxyvitamin D_3. Stimuli of endocrine organs not only increase hormonal release but also augment the formation of additional hormone. Thus, if *Phex* is needed for hormonal activation, its expression might also be elevated by a low phosphate diet.

Thus, our goals were first to determine the relative expression level of *Phex* in various tissues of normal mice and rats, and second to test whether the mRNA expression of *Phex* in any of these organs was sensitive to low phosphate diet.

Results

Phex Expression

We have measured the *Phex* mRNA expression level in a variety of mouse organs (Fig. 1). A constant amount of mRNA (0.8 μg) was subjected to reverse transcription (RT) and the polymerase chain reaction (PCR), so that the resulting PCR amplimer would reflect the mRNA level of *Phex* per μg total RNA in the tissue.

Note the wide disparity between organs. The *Y* axis of Fig. 1 is drawn on a log scale. The tissues are arbitrarily grouped into three clusters of strong, moderate, and weak expression that differed by approximately an order of magnitude from one to the next. The highest *Phex* expressions occurred in the gonads, brain, and lung. Moderate expression was observed at about 10% of the strong level for the thyroparathyroid complex, thymus, kidney, heart, calvaria, and skeletal muscle. Weak expression was recorded in other tissues at approx 10% of the moderate level and 1% of the strong level. These weakly expressing tissues included the pancreas, spleen, adrenal, uterus, duodenum, blood, liver, salivary gland, and femur. Note that of the two skeletal samples, the calvaria had a moderate expression and the femur had a weak expression.

Effect of Low Phosphate Diet

Mice

Genetically normal mice were fed a normal or low phosphate diet for 7 d to test for an increased *Phex* expression as a defensive mechanism against hypophosphatemia. The diet was effective in significantly lowering serum phosphate [2.78 ± 0.14 *(15)* vs 1.15 ± 0.07 *(16)* m*M*, normal vs low phosphate diet, mean ± SEM (*n*), $p < 0.001$] and significantly raising serum calcium [2.36 ± 0.02 *(15)* vs 2.68 ± 0.03 *(16)* m*M*, $p < 0.001$].

The response of *Phex* mRNA expression to a low phosphate diet is shown in Table 1. The tissues are listed in Table 1 in the same order as in Fig. 1. The values differ from Fig. 1 because these are ratios of *Phex* mRNA to glyceraldehyde-3-phosphate dehydrogenase (G3PDH) mRNA, whereas Fig. 1 was the *Phex* mRNA levels amplified from a constant amount of mRNA. Normalizing the expression of *Phex* to that of G3PDH was desirable to reduce the variation between samples caused by varying efficiency of the RT-PCR procedure. The G3PDH levels varied between organs (data not shown), and the level of the G3PDH primers was varied to optimize the *Phex* assay for each organ. A smaller amount of G3PDH primers was used with organs with weak *Phex* expression to allow both amplimers to be quantified. For these reasons the two sets of values (Fig. 1 and Table 1) are not identical.

In response to the low phosphate diet, the mRNA expression of *Phex* in most organs was not significantly affected (Table 1). There was a significant decrease in *Phex* expression in the spleen ($p = 0.002$). There was an increase in *Phex* expression in the thyroparathyroid gland complex that approached significance ($p = 0.05$). The components of this complex were too small to explore this effect further to identify the source of the increase. This led to the decision to examine a larger species.

Rats

Normal rats were fed the normal or low phosphate diet for 7 d. The low phosphate diet significantly lowered the plasma phosphate level (Table 2).

A variety of small organs were examined for a possible change in *Phex* expression. The pituitary gland had a significantly increased *Phex* expression in response to the low phosphate diet (Fig. 2). Adjacent areas of the hypothalamus were not significantly affected (Table 2). Representative gels are shown in Fig. 3 of the pituitary (top of Fig. 3) and hypothalamus (bottom of Fig. 3). The increased brightness of the *Phex* band from the pituitary gland of rats that were fed the low phosphate diet is apparent.

The thyroparathyroid complex was dissected to separate the parathyroids, the central region of the thyroid near the parathyroid [which is thought to be the source of calcitonin *(21,22)* (thyroid 1)], and the remainder of the thyroid (thy-

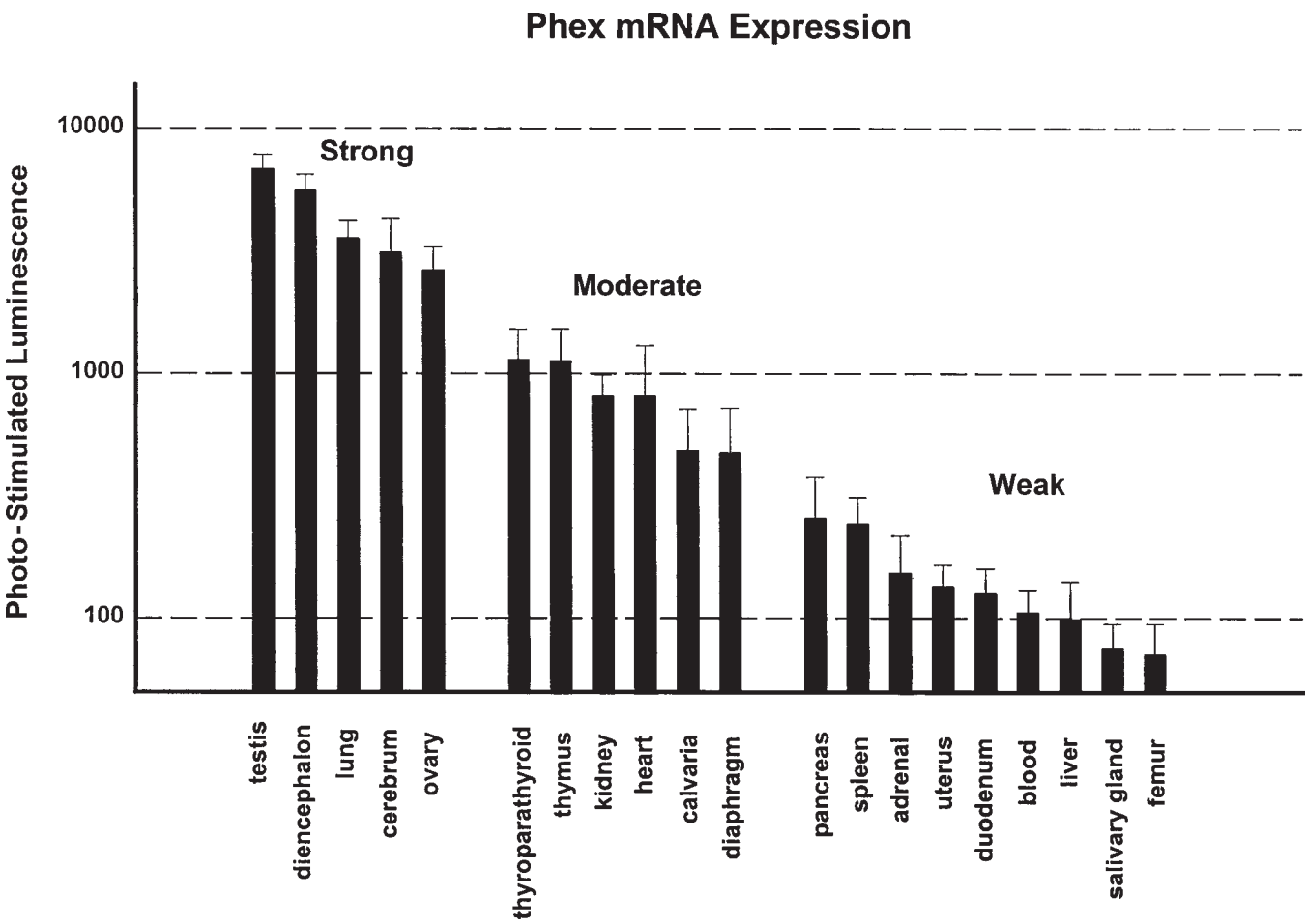

Fig. 1. *Phex* mRNA expression in tissues of normal mice. Data are mean ± SEM for 5–17 observations per group. Data are the ^{32}P levels measured in units of photo-stimulated luminescence. Radioactively labeled oligonucleotide probes were hybridized to Southern blots of *Phex* amplimers from PCR of constant amounts of mRNA (0.8 μg). Means are ranked in descending order and are arbitrarily grouped into clusters of strong, moderate and weak expression. Note the log scale on the *Y* axis. The lowest activity levels approached the limits of detection of the assay.

roid 2). None of these three areas had a significant change in *Phex* expression due to the low phosphate diet. The adrenal glands and atrium of the heart were also not significantly affected by the low phosphate diet.

While all of the tissues tested gave a reliable and reproducible amplimer of *Phex*, the G3PDH amplimer was not universally detectable in the two regions of the thyroid and in the atrium of the heart. For this reason, these data are reported as the radioactivity level of the hybridized *Phex* amplimer in Table 2 whereas the other tissues are reported as the radioactivity of the *Phex* amplimer normalized to the radioactivity of the G3PDH amplimer (Table 2).

Discussion

We report here the widespread expression of *Phex* in a variety of organs in growing mice and rats. There is relatively strong expression in brain, lung, testis, and ovary. In contrast, skeletal tissue has relatively little *Phex* expression with lower levels in calvaria and almost undetectable levels in femur.

We have found that the expression of *Phex* decreases with age in some organs in the mouse *(23)*. While the expression of *Phex* changes little with age in brain, it falls markedly in bone, lung, and kidney as the animals get older *(23)*. Embryonic tissues seem to express *Phex* at a higher level *(24)*. This high expression of *Phex* in embryonic bone has suggested a local role for the gene in bone *(25)*. In addition, in growing animals, we report herein the widespread expression of *Phex* in several organs apparently unrelated to bone and phosphate metabolism. This would suggest possible roles for *Phex* in a variety of organs so that its activity may not be focused exclusively on bone and bone minerals.

It is not at all clear how mutations of *Phex* cause abnormalities in the kidney and skeleton while leaving most other organs seemingly unaffected. For example, high *Phex* levels were found in brain, but there is no evidence for abnor-

Table 1
Effect of Low Phosphate Diet on *Phex* Expression in Mouse Tissues[a]

Tissue	Normal P Diet	Low P Diet	Ratio	Probability
Testes	561 ± 40	604 ± 73	1.06 ± 0.07 (7)	NS
Diencephalon	464 ± 69	512 ± 83	1.10 ± 0.13 (10)	NS
Lung	743 ± 134	589 ± 131	0.87 ± 0.12 (17)	NS
Cerebrum	499 ± 113	459 ± 72	1.05 ± 0.15 (6)	NS
Ovary	579 ± 95	624 ± 184	0.99 ± 0.15 (6)	NS
Thyroparathyroid	772 ± 126	1210 ± 225	2.04 ± 0.49 (15)	$p = 0.05$
Thymus	155 ± 48	159 ± 69	1.29 ± 0.17 (17)	NS
Kidney	215 ± 64	216 ± 52	1.21 ± 0.19 (11)	NS
Heart	687 ± 271	511 ± 180	0.97 ± 0.17 (6)	NS
Calvaria	201 ± 123	171 ± 69	1.02 ± 0.39 (6)	NS
Diaphragm	865 ± 819	417 ± 340	0.75 ± 0.20 (3)	NS
Pancreas	256 ± 172	340 ± 254	1.74 ± 0.81 (8)	NS
Spleen	445 ± 119	266 ± 92	0.60 ± 0.10 (12)	$p = 0.002$
Adrenal	110 ± 30	125 ± 48	1.45 ± 0.42 (12)	NS
Uterus	129 ± 33	102 ± 34	0.91 ± 0.34 (8)	NS
Duodenum	19 ± 3	17 ± 3	1.01 ± 0.29 (5)	NS
Blood	41.0 ± 9.5	49.9 ± 18.7	1.08 ± 0.14 (15)	NS
Liver	143 ± 37	172 ± 61	2.25 ± 0.97 (6)	NS
Salivary gland	309 ± 109	338 ± 111	1.37 ± 0.37 (6)	NS
Femur	45 ± 27	52 ± 40	1.13 ± 0.43 (6)	NS

[a]The values are *Phex* mRNA expression as percent of G3PDH mRNA expression for tissues from mice fed the two diets. The tissues are listed in the same order as Fig. 1, but the values differ as this table is the *Phex*/G3PDH ratio. Data are mean ± SEM (*n*) for the number of paired samples. Probability tests the null hypothesis that the ratio = 1.00.

Table 2
Effect of Low Phosphate Diet on *Phex* Expression in Rat Organs and Plasma Phosphate Levels[a]

Tissue	Units	Normal P diet	Low P diet	Probability
Plasma P	m*M*	3.06 ± 0.06 (32)	1.70 ± 0.07 (33)	$p < 0.01$
Hypothalamus	% G3PDH	970 ± 116 (16)	1020 ± 135 (16)	NS
Adrenal	% G3PDH	598 ± 57 (11)	875 ± 170 (10)	NS
Parathyroid	% G3PDH	190 ± 76 (8)	123 ± 45 (7)	NS
Thyroid 1	PSL	972 ± 388 (16)	698 ± 326 (15)	NS
Thyroid 2	PSL	445 ± 184 (9)	490 ± 166 (8)	NS
Atrium of heart	PSL	2361 ± 670 (16)	3382 ± 910 (17)	NS

[a]The units are millimoles/liter for plasma phosphate, % of G3PDH mRNA for *Phex* mRNA for hypothalamus, adrenal and parathyroid; and PSL (units of radioactivity as photo-stimulated luminescence) for *Phex* mRNA for the two thyroid regions and for the atrium. Data are mean ± SEM (*n*).

mal functioning of the central nervous system in patients with mutations of *Phex (26)*. There is high expression of *Phex* in the gonads, but both hemizygous *Hyp* male and heterozygous and homozygous *Hyp* female mice are fertile *(2,4)*. There is high expression in the lungs, but gas transport is adequate to support a higher than normal metabolic rate in the *Hyp* mice *(27)*.

We describe the brain, gonads, and lung as having strong expression of *Phex*. Strong is used in relative terms since RT-PCR was used to identify *Phex* mRNA expression in the tissues. Northern blots and RNase protection assays have in general failed to detect *Phex* expression in adult tissues *(8,10,24)*. Our assay is specific for *Phex* since the M3/M6 primer pair is in the region of the gene deleted in the *Hyp* mice *(11)*, and, in fact, this amplimer is indeed missing in samples taken from the *Hyp* mouse *(12)*. The amplimer crosses an intron border so that the amplimer studied here is specific for RNA and is not inflated by contamination with genomic DNA. Also, we have shown that the measurement of *Phex* expression, normalized to G3PDH, is linear over the range of expression studied here with a standard error of the estimate of ± 12% *(23)*. Each observation was repeated many times with independent animals to reduce the variability in the mean value. This gives us

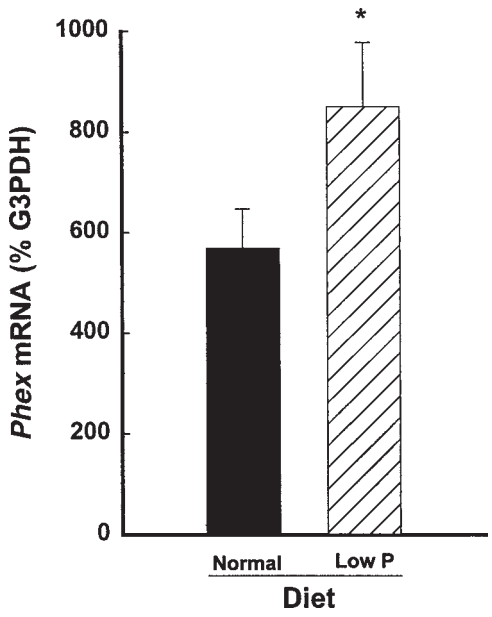

Fig. 2. Effect of low P diet (striped bar) on *Phex* expression by the pituitary gland of the normal rat. Data are mean ± SEM for 24–25 observations per group. * denotes $p = 0.03$ in comparison to the normal diet group (filled bar).

confidence that the data reported herein reflect mRNA levels for the *Phex* gene.

We have hypothesized for many years that *Phex* plays a key role in the hormonal regulation of phosphate in the extracellular fluid of vertebrates *(17)*. Mutations of *Phex* in humans *(1)* and mice *(2,3)* lead to hypophosphatemia due to reduced renal retention of phosphate. The animals *(5,28)* and patients *(6)* fail to respond to their hypophosphatemia by either raising the renal reabsorption of phosphate or by increasing plasma 1,25-dihydroxyvitamin D_3. This suggests that mutations of *Phex* inactivate a homeostatic mechanism for the regulation of plasma phosphate levels.

Parabiosis *(17,18)* and renal transplantation *(19)* both suggest a humoral origin for these abnormalities. These studies suggest the presence in mammals of a hormonal system for the regulation of phosphate levels. *Phex* is a member of a family of membrane-bound neutral endopeptidases, which activate or inactivate humoral factors *(15)*. These data suggest that *Phex* either activates a phosphate-conserving hormone or inactivates a phosphaturic hormone. What this hormone is, where it comes from, and how its secretion is controlled are all unknown. We undertook the present experiments to attempt to identify organs and tissues sensitive to the administration of a low phosphate diet. Stimuli of endocrine secretion are known to enhance the synthetic pathway for the hormone as well as enhancing hormonal secretion. We anticipated that an organ that participated in the defense against hypophosphatemia would have an increase in *Phex* expression when confronted with the challenge of reduced plasma phosphate. Only the pituitary gland gave such a response. Further work must be done to understand this change in the pituitary. It may represent the site of synthesis of a phosphate-regulating hormone, or it may be an unrelated physiological response to the drop in circulating phosphate levels.

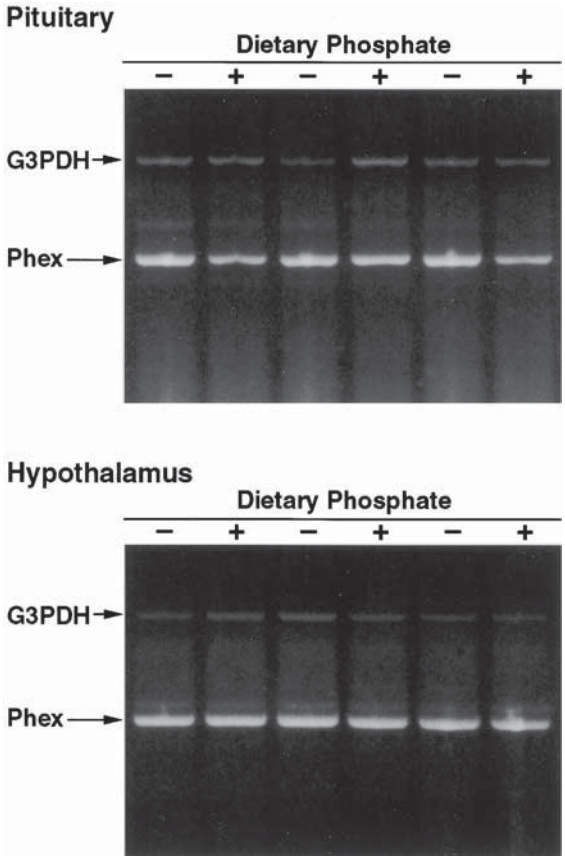

Fig. 3. Ethidium bromide staining of two agarose gels following electrophoresis. Amplimers of *Phex* and G3PDH are shown from the pituitary **(top)** and hypothalamus **(bottom)** of rats fed a normal phosphate (+) or a low phosphate (–) diet. Note that for the pituitary gland, the brightness of the *Phex* band is intensified in animals fed the low phosphate diet. The expression of G3PDH in the pituitary was unaffected by the low phosphate diet, and neither *Phex* nor G3PDH was affected in the hypothalamus.

It is intriguing that a recent report by Mulroney et al. *(29)* found that a low phosphate diet up-regulated the expression of the sodium-phosphate transporter NaPi-2 in the kidney and hypothalamus of rats. When phosphate was infused into the third ventricle of the brain, there was decreased NaPi-2 expression in the hypothalamus as well as in the kidney *(29)*. These tantalizing suggestions for a role for the hypothalamus and pituitary in the regulation of phosphate homeostasis deserve further exploration.

Attempts have been made to isolate this humoral agent from the blood of *Hyp* mice *(20)*. After partial purification, there appears to be a proteinacious material associated with a single peak on HPLC in the Hyp mice that inhibited phosphate transport in isolated OK cells *(20)*.

The mouse spleen had a significant reduction in *Phex* expression when the animal was fed a low phosphate diet. The low probability value (0.002) gives confidence in the reliability of the conclusion. There is no apparent reason for this change. Further exploration is needed to evaluate the significance of this finding. The mouse thyroparathyroid complex had a change with the low phosphate diet that was of borderline significance ($p = 0.05$). It is a small tissue that was at the limit of sensitivity of our method for quantification. The inability to confirm this observation with the larger rat thyroparathyroid complex suggests that the mouse observation may have been a random false-positive result.

In summary, *Phex* is expressed in a variety of hard and soft tissues in mice and rats at widely variable levels. The organs with the strongest expression levels in adult animals seem unrelated to skeletal or phosphate metabolism. The levels of *Phex* expression in most tissues is unresponsive to the feeding of a low phosphate diet. There was a significant increase in *Phex* expression in the pituitary gland that warrants further exploration to determine its role in phosphate conservation.

Materials and Methods

Mice

Normal male and female mice were raised on the B6C3H hybrid background as previously described *(4)*. At 6 wk of age, they were fed a normal phosphate diet (1.0% P, 1.0% Ca, Teklad 86129, lot 391829, Harlan, Madison, WI) or a low phosphate diet (0.03% P, 1.0% Ca, Teklad 86128, lot 391828) with deionized water for 7 d. On the last day, the mice were weighed, anesthetized, exsanguinated *(5)*, and dissected to collect a variety of organs for analysis (Fig. 1). The tissues were frozen in liquid nitrogen and stored at –80°C until analyzed. A brain sample consisting of the cerebrum and underlying diencephalon with the attached pituitary gland was removed. The cerebrum and diencephalon were divided and assayed separately. Serum levels of total calcium *(30)* and inorganic phosphate *(31)* were measured. All animal work was done in an AAALAC accredited vivarium under protocols approved by the Institutional Animal Care and Use Committee.

Rats

Sprague-Dawley female rats (135 ± 1 g) were placed on the normal phosphate or low phosphate diet as described above for 7 d with deionized water. Rats were anesthetized with isoflurane, one tube of blood was collected from the orbital sinus for plasma phosphate assay *(31)*, and various tissues were collected (Table 2 and Fig. 3) as described above. The pituitary and adjacent hypothalamus were processed separately. The parathyroid glands, the central region of the thyroid adjacent to the parathyroids [thought to be the source of calcitonin *(21,22)* (thyroid 1)] and the remainder of the thyroid (thyroid 2) were collected and processed separately.

Phex mRNA Expression

Tissues were homogenized with a Polytron sonicating homogenizer (PT10/35, Brinkman Instrument Inc., Westbury, NY), and total RNA was extracted with TRIzol (Gibco-BRL, Gaithersburg, MD). The RNA samples (0.8 μg) were then reverse transcribed at 42°C for 15 min using random hexamers as primers. This was then amplified by 35 cycles of PCR (GeneAmp RNA PCR kit, N808-0143 Perkin Elmer, Foster City, CA) with primers for both *Phex* and G3PDH. PCR was done with a GeneAmp PCR System 2400 (Perkin Elmer). The cycling conditions were 95°C for 2 min; 80°C for 5 min (with hot-start addition of the Taq DNA polymerase); 35 cycles of 95°C (15 s) and either 55°C (mouse) or 62°C (rat) for 30 s; and a final extension at 72°C for 7 min. The resulting amplimers were separated by electrophoresis on a 1.5% agarose gel in 0.5X TBE with ethidium bromide at 100 V *(32)*. Lane 1 of the gels contained 1.0 μg of a 100 base pair (bp) DNA Ladder (cat. no. 15628-019, Gibco-BRL) as a molecular weight (MW) marker. For all assays, the same tissues from animals of alternating normal and low phosphate diet were run in adjacent lanes of the electrophoretic gels.

The M3/M6 primer set described by Du et al. *(13)* for mouse *Phex* was used (Keystone Laboratories, Menlo, CA). This amplified a portion of the 3' end of the message with a predicted amplimer size of 627 bp. For the rat tissues, PCR primers were constructed from GenBank Accession No. AJ001637 with the forward sense primer (ACAA CAACTCTGCTGCCTCAG) beginning at position 1667 and extending 479 bp through the reverse antisense primer (AGCTCTCCAGCAGGAAATCGT). The primer pairs were added to the PCR mixture in a final concentration of 0.15 μ*M* for *Phex* and 0.009 or 0.019 μ*M* for G3PDH. G3PDH was amplified as a positive control in all samples with primers from Clontech (Palo Alto, CA). The G3PDH primer set amplifies a 983 bp fragment.

Blotting and Hybridization

Southern blot hybridization was performed on the amplified samples. The agarose gels were blotted to nylon membranes (Hybond-N+, Amersham, Arlington Heights, IL) *(32)*, UV cross-linked, and hybridized with Rapid-hyb buffer (Amersham) at 42°C using γ-^{32}P ATP end-labeled oligonucleotides internal to the PCR primer pairs for *Phex* and G3PDH (Clontech) as the probes *(32)*. The oligonucleotide M4 *(13)* was used for the mouse, and a sense internal oligonucleotide (CGAAAGTGCCCTCCCTTATGT) was used for the rat. The membranes were washed in 6X SSC, 0.1% SDS; 4X SSC, 0.1% SDS; and 2X SSC, 0.1% SDS for consecutive 15 min washes at 25°C. The radioactivity levels of the Southern blots were quantified with a phosphor imager, Fujifilm Bio-Imaging Analyzer BAS-1500 with MacBAS Ver. 2.4 software. Rectangles of constant area were defined encompassing the *Phex* or G3PDH activity in each lane of the Southern blot. Radioactivity was recorded in units of photo-stimulated luminescence (PSL). Back-

ground was subtracted from each area, and the *Phex*/G3PDH ratio was calculated for each lane of each blot. The ratios as well as the *Phex* and G3PDH data were subjected to statistical analysis. Earlier quality control studies indicated that the 627 bp amplimer was from mouse *Phex (12)*.

Statistics

Individual treatment groups are presented as mean ± SEM. To test for differences in *Phex* expression in tissues from animals fed normal and low phosphate diets, two different methods were used to control for the variation between blots. For the mice, pairs of samples (one from each of the two diets) were run in duplicate in adjacent lanes of the gels. The duplicate samples were averaged and treated as one observation. The ratio of *Phex* expression between tissues from animals on the low phosphate diet and tissues from animals on the normal phosphate diet was calculated for each pair. Possible significant effects of the low phosphate diet were tested by the *t*-test *(33)*. For the rats, PCR amplimers from tissues of animals on the normal and low phosphate diets were run on each gel. The data from each blot were treated as a statistical block, and the variation between the blocks was controlled by factorial analysis of variance (ANOVA) techniques. Computations were done with SPSS/PC + (V 3.0, SPSS Inc., Chicago, IL).

The strategy was to measure *Phex* expression in an initial set of animals on a normal or low phosphate diet and to compare the results. Tissues with responses less than 1 SEM were not studied further. Additional animals were done for other organs until the results either became significant or fell below 1 SEM. This accounts for the uneven replication shown in Tables 1 and 2.

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