Adenoviral expression of NHERF-1 in NHERF-1 null mouse renal proximal tubule cells restores Npt2a regulation by low phosphate media and parathyroid hormone

Rochelle Cunningham,1 Deborah Steplock,1 Xiaofei E,1 Rajat S. Biswas,1 Fengying Wang,1 Shirish Shenolikar,4 and Edward J. Weinman1,2,3

1Department of Medicine, 2Department of Physiology, University of Maryland School of Medicine, and 3Medical Service, Department of Veterans Affairs Medical Center, Baltimore, Maryland; and 4Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina

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The reabsorption of filtered phosphate in the proximal convoluted tubule of the kidney is mediated, in large measure, by the apical membrane sodium-dependent phosphate transporter 2a (Npt2a, NaPi IIa) (2, 12). In response to a low-phosphate diet or incubation in low phosphate media, Npt2a is recruited to the brush-border membrane of renal proximal tubule cells where it mediates increased sodium-dependent phosphate uptake (4, 10, 16, 17, 25). Parathyroid hormone (PTH), on the other hand, facilitates the retrieval of Npt2a from the brush-border membrane and increases the urinary excretion of phosphate (1, 5, 16). Recent experiments have demonstrated that specific proteins that interact with the COOH terminus of Npt2a have significant effects on its trafficking (3, 7, 9, 18, 19). The best studied of these associated proteins is the sodium-hydrogen exchanger regulatory factor-1 (NHERF-1), a PDZ domain-containing adaptor that binds to the COOH terminus of Npt2a (7, 9, 19, 22, 23, 26). Inactivation of the NHERF-1−/− gene in mice results in a decrease in the serum concentration of phosphate, an increase in the urinary excretion of phosphate, and a decrease in the renal proximal tubule brush-border membrane abundance of Npt2a (18). In response to culture in low phosphate media, wild-type renal proximal tubule cells but not NHERF-1−/− cells recruit Npt2a to the plasma membrane resulting in increased sodium-dependent phosphate transport (4). PTH decreases brush-border membrane expression of Npt2a and phosphate transport in wild-type proximal tubule cells but does not affect either parameter in NHERF-1−/− cells (5). Although these cellular studies suggest a potential role for NHERF-1 in regulating Npt2a function, the specific contribution of NHERF-1 in the physiological regulation of Npt2a in the apical membrane of renal proximal tubule cells has not been investigated.

To date, more than 40 proteins including many G protein-coupled receptors and other signaling proteins have been shown to bind NHERF-1 (19). The present studies were designed to differentiate between the direct effects of NHERF-1 compared with the indirect effects on other signaling pathways and/or other developmental alterations in renal tissues elicited by absence of this multifunctional protein on basal and regulated phosphate transport. Specifically, we used cultured renal proximal tubule cells from wild-type and NHERF-1−/− null cells and viral-mediated gene transfer to restore NHERF-1 expression in the mutant NHERF-1−/− proximal tubule cells and demonstrate the recovery of Npt2a regulation by incubation in low phosphate media and by PTH. These studies highlight the key role played by NHERF-1 in transducing critical signals elicited by multiple physiological stimuli to regulate Npt2a function in the mammalian kidney.

METHODS

Animals and preparation of renal proximal tubule cells. Male NHERF-1−/− mice (B6.129-Sle9a3r1hsos1/Sld) bred into a C57BL/6 background for six generations and parental wild-type inbred control
C57BL/6 mice age 12 to 16 wk were used in the current experiments (18). To prepare primary renal proximal tubule cell cultures, mice were euthanized by intraperitoneal injection of 100 mg/kg pentobarbital sodium followed by decapitation. The kidneys were removed, and the cortices were dissected, minced, and digested using 1% collagenase type II (Worthington) and 0.025% soy bean trypsin inhibitor, and sedimented on 45% Percoll (4, 5). The proximal tubule cells were grown in an incubator at 37°C in 5% CO2 in DMEM-F12 media containing 50 U/ml penicillin, 50 μg/ml streptomycin, 10 ng/ml epidermal growth factor, 0.5 mM hydrocortisone, 0.87 μM bovine insulin, 50 μM proglandlin E1, 50 nM sodium selenite, 5 μg/ml human transferrin, and 5 μM thrombin. At 5 to 7 days after plating, no attempt was made to pass the cells. Media were replaced every 2 days until the cells achieved confluence and the cortices were dissected, minced, and digested using 1% collagenase type II (Worthington) and 0.025% soy bean trypsin inhibitor, and sedimented on 45% Percoll (4, 5). The proximal tubule cells were washed three times and preincubated for 5 min in nontransport medium containing 137 mM NaCl, 5.0 mM KCl, 1.0 mM CaCl2, 1.8 mM MgSO4, and 0.1 mM KH2PO4. Phosphate uptake was initiated by the addition of transport medium containing 32P-labeled phosphate (4, 5). Sodium-dependent phosphate uptake averaged 4.9 ± 0.01 nmol·mg protein−1·10 min−1 in NHERF-1 null cells infected with adenovirus-GFP and 6.9 ± 1.1 in cells infected with adenovirus-GFP-NHERF-1 (P < 0.01, n = 9). As shown in Fig. 1A, there was a significant increase in Npt2a abundance in the plasma membranes from adenovirus-GFP-NHERF-1-infected NHERF-1−/− cells in the plasma membranes from adenovirus-GFP-NHERF-1-infected NHERF-1−/− cells. In six separate experiments, there was an average increase of 40.0 ± 9.9% (P < 0.01) in plasma membrane content of Npt2a in adenovirus-GFP-NHERF-1-infected cells compared with control adenovirus-GFP-infected cells. Western immunoblot analyses of whole cell lysates (Fig. 1B) indicated no difference between NHERF-1 null cells infected with adenovirus-GFP or adenovirus-GFP-NHERF-1. The sense primer was 5′-CTTCAACATCCTGGGATTTACTG and the anti-sense primer was 5′-TAGAGCCGGTTGGGTG-3′. For 15S RNA, the sense and anti-sense primers were 5′-GCAAATTATTCCTCCATGAAGC-3′ and 5′-GGGCTGCTACA- AACCATCACA-3′, respectively. PCR reactions in a volume of 25 μl, containing 400 nM gene-specific primers, were performed using iQ SYBR Green Supermix (Bio-Rad). Following activation of iTaq DNA polymerase (2 min at 94°C), the samples were amplified for 45 cycles at 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s with a final extension of 72°C for 2 min. Relative expression was calculated according to the iCycler IQ Real-Time PCR Detection System Manual. 

**RESULTS**

In initial experiments, we examined the effect of infection of NHERF-1 null cells with either adenovirus-GFP or adenovirus-GFP-NHERF-1. Cells were exposed to virus for 24 h and studies were undertaken 24 h after removal of the viral particles. Sodium-dependent phosphate uptake averaged 4.9 ± 0.8 nmol·mg protein−1·10 min−1 in NHERF-1−/− cells infected with control adenovirus-GFP and 6.9 ± 1.1 in cells infected with adenovirus-GFP-NHERF-1 (P < 0.01, n = 9). As shown in Fig. 1A, there was a significant increase in Npt2a abundance in the plasma membranes from adenovirus-GFP-NHERF-1-infected NHERF-1−/− cells. In six separate experiments, there was an average increase of 40.0 ± 9.9% (P < 0.01) in plasma membrane content of Npt2a in adenovirus-GFP-NHERF-1-infected cells compared with control adenovirus-GFP-infected cells. Western immunoblot analyses of whole cell lysates (Fig. 1B) indicated no difference between NHERF-1 null cells infected with adenovirus-GFP or adenovirus-GFP-NHERF-1 (lane 2) using antibodies specific for Npt2a (A and B), GFP (C), or NHERF-1 (D). A, C, and D: plasma membrane fraction of the cells. B: whole cell lysates. Molecular weight markers (kDa) are shown.
fectected with adenovirus-GFP and adenovirus-GFP-NHERF-1 cells (percent difference 0.3 ± 0.33%, \( P = \) not significant, \( n = 6 \)). Using quantitative RT-PCR, Npt2a mRNA relative to 15S mRNA was not different in NHERF-1/−/− cells infected with adenovirus-GFP-NHERF-1 compared with adenovirus-GFP-infected cells (data not shown). With the use of an anti-GFP antibody, NHERF-1 expression in the plasma membrane fraction was readily detected in adenovirus-GFP-NHERF-1-infected cells but not in adenovirus-GFP-infected cells (Fig. 1C). The identity of GFP band was confirmed using an antibody to NHERF-1 (Fig. 1D). Figure 2 is representative confocal microscopic images of NHERF-1/−/− cells showing the presence of diffuse cellular Npt2a staining in cells infected with adenovirus-GFP but the presence of Npt2a on the surface of cells infected with adenovirus-GFP-NHERF-1.

Our prior studies indicated that, by contrast to wild-type proximal tubule cells, NHERF-1 null cells grown in low phosphate media did not increase sodium-dependent phosphate transport or the plasma membrane content of Npt2a and were resistant to the inhibitory effects of PTH (4, 5). We next determined whether reexpression of NHERF-1 in NHERF-1 null cells could restore these adaptive responses. Where indicated, half of each individual preparation of NHERF-1/−/− proximal tubule cells was infected using the control adenovirus-GFP and the other half infected using adenovirus-GFP-NHERF-1. To minimize possible differences in the levels of expression between different cultures, cells from the same preparation infected with either adenovirus-GFP or adenovirus-GFP-NHERF-1 were studied following incubation in low (0.1 mM) or high (1.9 mM) phosphate media or in the presence or absence of PTH. Sodium-dependent phosphate uptake averaged 4.1 ± 0.2 nmol·mg protein\(^{-1}\)·10 min\(^{-1}\) and 4.1 ± 0.3 (\( P = \) not significant, \( n = 6 \)) in null cells infected with adenovirus-GFP grown in low- and high-phosphate media, respectively (Table 1). By contrast, sodium-dependent phosphate uptake averaged 7.4 ± 0.6 nmol·mg protein\(^{-1}\)·10 min\(^{-1}\) in NHERF-1 null cells infected with adenovirus-GFP-NHERF-1 grown in low phosphate media (\( P < 0.01 \) vs. adenovirus-GFP-infected NHERF-1/−/− cells grown in the same low phosphate media). In response to growth in high phosphate media, adenovirus-GFP-NHERF-1-infected NHERF-1 null cells demonstrated a significantly lower rate of sodium-dependent phosphate transport of 5.4 ± 0.3 nmol·mg protein\(^{-1}\)·10 min\(^{-1}\) (\( P < 0.01 \)). There was no significant difference in plasma membrane expression of Npt2a in adenovirus-GFP-infected NHERF-1 null cells incubated in low- or high-phosphate media (% difference = 11.5 ± 8.4%, \( P = \) not significant, \( n = 4 \)). Adenovirus-GFP-NHERF-1-infected cells grown in low-phosphate media had a 28.2 ± 2.5% greater abundance of Npt2a in the plasma membrane compared with cells grown in high-phosphate media (\( P < 0.05 \); Fig. 3). The relative abundance of GFP-NHERF-1 in the plasma membrane was minimally but not statistically significantly higher in cells grown in low- vs. high-phosphate media (10.2 ± 3.5%, \( P = \) not significant). These experiments established the ability of NHERF-1 to restore the forward trafficking of Npt2a to the apical membrane to increase sodium-dependent phosphate transport in response to growth in low phosphate media.

NHERF-1/−/− cells are resistant to the inhibitory effects of PTH (5). Accordingly, we examined whether expression of NHERF-1 in NHERF-1 null cells could restore the response to the hormone. Sodium-dependent phosphate uptake averaged 5.7 ± 0.5 nmol·mg protein\(^{-1}\)·10 min\(^{-1}\) in the absence and 5.8 ± 0.4 in the presence of PTH (\( P = \) not significant, \( n = 7 \); Table 2) in null cells infected with adenovirus-GFP. Sodium-dependent phosphate uptake averaged 9.5 ± 0.8 nmol·mg protein\(^{-1}\)·10 min\(^{-1}\) in NHERF-1 null cells infected with adenovirus-GFP-NHERF-1 (\( P < 0.01 \) vs. adenovirus-GFP-infected NHERF-1/−/− cells). In response to PTH, adenovirus-GFP-NHERF-1-infected NHERF-1 null cells demonstrated a significantly lower rate of sodium-dependent phosphate transport of 7.4 ± 0.5 nmol·mg protein\(^{-1}\)·10 min\(^{-1}\) (\( P < 0.01 \), \( n = 7 \)).

There was no significant difference in the abundance of Npt2a in the plasma membrane in adenovirus-GFP-infected NHERF-1 null cells treated with PTH compared with control cells (% difference = −1.0 ± 6.9, \( n = 4 \), \( P = \) not significant).

Table 1. Effect of growth in low- or high-phosphate media on sodium-dependent phosphate transport in mouse NHERF-1/−/− proximal tubule cells infected with adenovirus-GFP or adenovirus-GFP-NHERF-1

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<th>Adenovirus-GFP</th>
<th>Adenovirus-GFP-NHERF-1</th>
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<tr>
<td>Low Phosphate</td>
<td>4.1±0.2</td>
<td>7.4±0.6(*)</td>
</tr>
<tr>
<td>High Phosphate</td>
<td>4.1±0.3</td>
<td>5.4±0.3(†)</td>
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Results are expressed as means ± SE. The results of 6 experiments are summarized. Sodium-dependent phosphate transport (nmol·mg protein\(^{-1}\)·10 min\(^{-1}\)) was determined in NHERF-1/−/− cells infected with adenovirus-GFP or adenovirus-GFP-NHERF-1 incubated in either low-phosphate (0.1 mM) or high-phosphate (1.9 mM) media for 24 h. *\( P < 0.01 \) vs. adenovirus-GFP-infected cells. †\( P < 0.01 \) vs. adenovirus-GFP-NHERF-1-infected cells grown in low-phosphate media.

Fig. 2. Confocal microscopy images of NHERF-1/−/− cells infected with adenovirus-GFP (left) or adenovirus-GFP-NHERF-1 (right). Cells were stained with antibody to Npt2a.
of the inability of downstream second messengers to regulate response to PTH seen in the NHERF-1 null cells was the result compared with wild-type animals, suggesting that the lack of proximal convoluted tubule cells from NHERF-1 null mice messengers cAMP and diacylglycerol (13, 14). In our prior PTH1 receptor to modulate PTH signaling via the second

Plasma membrane abundance of GFP-NHERF-1 was 13.9 ± 0.05) lower plasma membrane expression of Npt2a (Fig. 4).

-PTH +PTH

By contrast, the decrease in sodium-dependent transport of phosphate in PTH-treated adenosivirus-GFP-NHERF-1-infected NHERF-1 -/− cells was associated with a 29.6 ± 4.9% (P < 0.05) lower plasma membrane expression of Npt2a (Fig. 4). Plasma membrane abundance of GFP-NHERF-1 was 13.9 ± 4.7% lower in NHERF-1 null cells infected with adenosivirus-GFP-NHERF-1 cells treated with PTH compared with non-PTH-treated cells (P = not significant, n = 4).

PTH is known to signal via the generation of cAMP leading to PKA activation and by the activation of protein kinase C (4, 5, 20). Prior cellular studies showed that NHERF-1 binds to the PTH1 receptor to modulate PTH signaling via the second messengers cAMP and diacylglycerol (13, 14). In our prior studies, we found no apparent differences in PTH signaling in proximal convoluted tubule cells from NHERF-1 null mice compared with wild-type animals, suggesting that the lack of response to PTH seen in the NHERF-1 null cells was the result of the inability of downstream second messengers to regulate

Table 2. Effect of PTH on sodium-dependent phosphate transport in mouse NHERF-1 -/− proximal tubule cells infected with adenosivirus-GFP or adenosivirus-GFP-NHERF-1

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<th>Adenosivirus-GFP</th>
<th>Adenosivirus-GFP-NHERF-1</th>
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<tr>
<td>−PTH</td>
<td>5.7±0.5</td>
<td>9.5±0.8*</td>
</tr>
<tr>
<td>+PTH</td>
<td>5.8±0.4</td>
<td>7.3±0.5†</td>
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Results are expressed as means ± SE. The results of 6 experiments are summarized. Sodium-dependent phosphate transport (nmol·mg protein −1·10 min −1) was determined in the absence [parathyroid hormone (−PTH)] or presence of PTH (−10 M) in NHERF-1 -/− cells infected with adenosivirus-GFP or adenosivirus-GFP-NHERF-1. *P < 0.01 vs. adenosivirus-GFP-infected cells. †P < 0.01 vs. adenosivirus-GFP-NHERF-1-infected cells studied in the absence of PTH.

Table 3. Effect of cAMP and DOG on sodium-dependent phosphate transport in mouse wild-type and NHERF-1 -/− proximal tubule cells (% decrease from control)

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<tr>
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<th>cAMP</th>
<th>DOG</th>
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<tr>
<td>Wild-type (n = 6)</td>
<td>24.3±1.5*</td>
<td>28.9±2.6*</td>
</tr>
<tr>
<td>NHERF-1 -/− (n = 6)</td>
<td>−6.5±6.5</td>
<td>−7.3±7.7</td>
</tr>
<tr>
<td>NHERF-1 -/− infected with adenosivirus-GFP (n = 4)</td>
<td>−13.5±6.5</td>
<td>−10.9±5.1</td>
</tr>
<tr>
<td>NHERF-1 -/− infected with adenosivirus-GFP-NHERF-1 (n = 5)</td>
<td>33.3±5.2*</td>
<td>34.6±6.0*</td>
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Results are expressed as the percent decrease compared with control ± SE. The number of experiments is indicated in parenthesis. Sodium-dependent phosphate transport was determined in wild-type cells, NHERF-1 -/− cells, uninfected NHERF-1 -/− cells, and NHERF-1 -/− cells infected with either adenosivirus-GFP or adenosivirus-GFP-NHERF-1 under control conditions or after incubation in 8-bromo-cAMP (100 μM) to activate PKA or DOG (10 μM) to activate protein kinase C for 45 min. *P < 0.01 vs. control cells.
presence of DOG (P = not significant; n = 6) in NHERF-1−/− cells. While infection of NHERF-1 null cells with adenovirus-GFP had no effect on the lack of response to cAMP or DOG, infection with adenovirus-GFP-NHERF-1 resulted in an inhibitory response to both cAMP (% decrease = 33.3 ± 5.2%, P < 0.05) and DOG (% decrease = 34.6 ± 6.0%, P < 0.05; Table 3). These data strongly suggest that NHERF-1 acts at postreceptor sites to transduce the second messenger signals by which PTH regulates Npt2a function in renal tissue.

DISCUSSION

Prior studies from our laboratory indicated that sodium-dependent phosphate transport in proximal tubule cells in primary culture from NHERF-1 null mice has a lower basal rate of transport, fails to upregulate phosphate transport in response to incubation in a low phosphate media, and is resistant to the inhibitory effect of PTH (4, 5). These studies suggest that NHERF-1 may play a key role in processes that both recruit and retrieve Npt2a from the apical membrane of proximal tubule cells. On the other hand, dysfunction of the mouse NHERF-1 gene was reported to induce significant structural alterations in gut epithelia, inducing malformation of microvilli (16). While analyses of renal epithelia in our NHERF-1 null mice failed to show similar structural alterations, the potential indirect impact on Npt2a function and regulation of the loss of NHERF-1 that binds and regulates a multitude of receptors, transporters, scaffolds, and signaling proteins could not be discounted (18). Thus the current experiments that employ adenovirus constructs to affect highly efficient expression of NHERF-1 in NHERF-1−/− renal proximal tubule cells are critical for assessing the direct functional impact of NHERF-1 expression on Npt2a localization and function. In initial experiments, we compared infection with control adenovirus-GFP with adenovirus-GFP-NHERF-1 and found that NHERF-1−/− proximal tubule cells expressing GFP-NHERF-1 had significantly higher rates of phosphate transport associated with increased Npt2a abundance in the brush-border membrane. This indicates that, in large measure, the defect in phosphate transport and Npt2a trafficking in NHERF-1−/− renal proximal tubule cells resides within these cells and is not the result of developmental defects or systemic factors associated with the absence of the NHERF-1 protein.

To fully understand the role of NHERF-1 in the physiological regulation of phosphate transport, we also examined the ability of NHERF-1 mutant cells to adapt phosphate transport in response to growth in low-phosphate media and to respond to PTH. As we previously reported and by contrast to wild-type proximal tubule cells, NHERF-1 null cells, herein infected with adenovirus-GFP, did not increase sodium-dependent phosphate transport or recruit Npt2a to the brush-border membrane in response to incubation in low-phosphate media (4). GFP-NHERF-1-expressing NHERF-1 null cells, on the other hand, demonstrated increased Npt2a abundance on the apical membrane and increased sodium-dependent phosphate transport in response to growth in low phosphate media. These findings provide direct experimental evidence that NHERF-1 plays a key role in regulating Npt2a trafficking in cultured proximal tubule cells in response to incubation in low-phosphate media and presumably following the feeding of a low-phosphate diet to intact animals (25). In cultured proximal tubule cells infected with GFP-NHERF-1 grown in low phosphate media, the increase in the plasma membrane abundance of Npt2a was not associated with a significant change in the abundance of GFP-NHERF-1. This finding is in accord with our prior observations in intact animals where feeding of a low-phosphate diet increased brush-border membrane abundance of Npt2a but not NHERF-1 (24). Taken together, these results indicate that within the sensitivity of these measurements, the recruitment of Npt2a to the plasma membrane is not obligatorily linked to the redistribution of NHERF-1. Accordingly, we favor the hypothesis that NHERF-1 functions as a membrane retention signal for Npt2a. Given the relative abundances of NHERF-1 and Npt2a, however, the current studies do not rule out the possibility that NHERF-1 could also function as a chaperone for the transporter.

Our current studies confirm that sodium-dependent phosphate transport in NHERF-1 null cells, by contrast to wild-type cells, is resistant to the inhibitory effect of PTH (5). Expression of NHERF-1 in NHERF-1 null cells results in the restoration of the inhibitory effect of PTH on sodium-dependent phosphate transport associated with a decrease in the plasma membrane abundance of Npt2a. Recent experiments using kidney slices from mice suggested that PTH decreases Npt2a abundance in brush-border membrane of the renal proximal tubule with lesser effects on the abundance of NHERF-1 (6). The present experiments would agree with these findings and indicate that treatment with PTH alters Npt2a abundance but does not significantly affect the abundance and/or distribution of GFP-NHERF-1 in NHERF-1−/− proximal tubule cells.

Studies in cultured cells showed that the NHERF proteins, in addition to binding to Npt2a, also bind the PTH1 receptor (PTH1R) to function as a molecular switch for downstream signaling (13, 14). When bound to NHERF-1, PTH1R preferentially signals through protein kinase C while its capacity to signal through cAMP is greatly decreased. The failure of PTH to inhibit phosphate transport in the NHERF-1 null cells, therefore, could arise from either the inability of PTH to regulate Npt2a or altered signaling of PTH1R. To address this question, we bypassed the PTH receptor and studied sodium-dependent phosphate transport in proximal cells treated with either cAMP to stimulate PKA or DOG to activate PKC. On average, PTH, cAMP, and DOG significantly inhibited sodium-dependent phosphate transport by 25 to 30% in wild-type cells but not in NHERF-1 null cells. Moreover, rescue of the NHERF-1 null cells with adenovirus-GFP-NHERF-1 restored the response to both cAMP and DOG, indicating that NHERF-1 was critical for the transduction of hormone signals mediated by both second messengers. Our prior biochemical studies had demonstrated no significant differences in the ability of PTH to increase cAMP levels or activate PKC in renal tissue from wild-type and NHERF-1 null mice (4, 5). When considered together, these data focus attention on the interaction between Npt2a and NHERF-1 rather than the interaction between NHERF-1 and PTH1R as the explanation for the resistance to PTH. This conclusion also fits the observed phenotype of the NHERF-1−/− mouse that manifests hypophosphatemia and increased excretion of phosphate (18). If the interaction between NHERF-1 and PTH1R were the primary defect, we would have predicted that null animals would excrete less phosphate and tend toward hyperphosphatemia, reflecting inactivation of the receptor. It is worth noting that
mouse proximal tubule cells express both NHERF-1 and NHERF-2 and that the relative abundance of NHERF-2 is not altered in the absence of a functional NHERF-1 gene (21). PTH1R appears to be able to use either NHERF-1 or NHERF-2 as the molecular switch while Npt2a shows greater selectivity for NHERF-1. Npt2a trafficking is defective in the absence of NHERF-1 despite the continued expression of NHERF-2 in mouse tissues (18).

In summary, the present experiments indicate a requirement for NHERF-1 in the regulation of phosphate transport and Npt2a surface expression in response to growth in a low-phosphate media and to PTH in primary renal proximal tubule cells. NHERF-1 null cells are resistant not only to PTH but also to the major second-message signaling pathways normally used by the PTH1 receptor, namely, PKA and PKC. Expression of GFP-NHERF-1 in NHERF-1 null cells restores the adaptive response to growth in low-phosphate media and the inhibitory responses to PTH, cAMP, and DOG. From these findings, we hypothesize that NHERF-1 complexes with Npt2a in the apical membrane of renal proximal tubule cells, an interaction necessary to stabilize or anchor Npt2a recruited to the plasma membrane in response to incubation in low-phosphate media. Moreover, we would suggest that NHERF-1/Npt2a complexes represent the major PTH-responsive pool of membrane Npt2a and that PTH, acting via PKA and/or PKC, regulates the disassembly of Npt2a/NHERF-1 complexes (6). The disassembly of the Npt2a/NHERF-1 complexes may, in turn, increase the lateral mobility of Npt2a in the apical membrane necessary to engage clathrin and other elements that result in the internalization and subsequent degradation of the transporter.

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