Calcium-sensing receptor regulation of PTH-inhibitable proximal tubule phosphate transport

Jianming Ba,1 Dennis Brown,2 and Peter A. Friedman1,3

Departments of 1Pharmacology and 2Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261; and 3Program in Membrane Biology and Renal Unit, Massachusetts General Hospital, Charlestown, Massachusetts 02129

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Ba, Jianming, Dennis Brown, and Peter A. Friedman. Calcium-sensing receptor regulation of PTH-inhibitable proximal tubule phosphate transport. Am J Physiol Renal Physiol 285: F1233–F1243, 2003. First published September 2, 2003; 10.1152/ajprenal.00249.2003.—Inorganic phosphate (Pi) is absorbed by proximal tubules through a cellular pathway that is inhibited by parathyroid hormone (PTH). The calcium-sensing receptor (CaSR) is expressed on apical membranes of proximal tubules. In the present studies, we determined the effect of luminal and/or basolateral PTH on phosphate absorption and tested the hypothesis that CaSR activation blocks PTH-inhibitable phosphate absorption. Single proximal S3 tubules were dissected from the kidneys of mice and studied by the Burg technique. Tubules were bathed with DMEM culture media supplemented with 6% BSA and perfused with an ultrafiltrate prepared from the bathing solution. 32P and FITC-inulin were added to the luminal perfusate to measure phosphate absorption (Jp,i) and fluid absorption (Jv), respectively. Jp,i averaged 2.9 pmol·min⁻¹·mm⁻² under control conditions and decreased by 20% upon addition of serosal PTH. PTH had no effect on Jv. Inclusion of PTH in the luminal perfusate reduced Jp,i to 2.1 pmol·min⁻¹·mm⁻². Combined addition of PTH to perfuse and bathing solutions reduced Jp,i to 1.5 pmol·min⁻¹·mm⁻² without affecting Jv. Indirect immunofluorescence studies revealed abundant PTH receptor (PTH1R) expression on brush-border membranes, with lower amounts on basolateral membranes. CaSRs were localized primarily, but not exclusively, to brush-border membranes. CaSR activation with luminal Gd³⁺ abolished the inhibitory action of PTH on Jp,i. Addition of Gd³⁺ to the serosal bathing solution had no effect on PTH-sensitive Jp,i. Gd³⁺ did not affect basal, i.e., PTH-independent Jp,i. Gd³⁺ had no effect on Jv when added to lumen or bath. Dopamine-inhibitable Jp,i was not affected by Gd³⁺. Experiments with proximal-like opossum kidney cells showed that elevated extracellular Ca²⁺ or NPS R467, a type IIa calciimimetic, inhibited PTH action on P₃ uptake. In conclusion, PTH1Rs are expressed on apical and basolateral membranes of mouse proximal tubules. Stimulating apical or basolateral PTH1R inhibits phosphate absorption. CaSR activation specifically regulates PTH-suppressible phosphate absorption.

RENAL PHOSPHATE REABSORPTION occurs mostly in proximal tubules through a cellular pathway. Type IIa Na-Pi cotransporters account for ~70% of brush-border membrane (BBM) Na-Pi cotransporter activity and are the target of parathyroid hormone (PTH) action (6). PTH inhibits inorganic phosphate (Pi) absorption by internalizing the apical BBM type Ia Na-Pi cotransporter (45). Functional type 1 PTH receptors (PTH1R) are located in both basolateral and, perhaps, on luminal membranes of proximal tubules (2). PTH internalizes the apical NaPi-IIa cotransporter in mouse kidney when added either to mucosal or basolateral surfaces of proximal straight tubules (64). Although PTH sequestered the Na-Pi cotransporter when applied to the luminal side of proximal tubules, the expression and functional role of apical PTH1Rs on phosphate absorption by intact segments of proximal tubules are not clear. The first goal of the present studies was to determine the effect of luminal PTH, alone or in combination with basolateral PTH, on phosphate absorption by single mouse proximal straight tubules and to localize the PTH1R.

Extracellular calcium influences renal phosphate absorption. Most studies report that elevating plasma calcium increases Pi absorption (1, 39, 54). Such an effect could arise indirectly by suppressing PTH release, or directly. Direct effects of calcium on proximal tubule Pi absorption have been examined only in a limited number of studies. In the first, eliminating luminal calcium decreased Pi absorption (24). Such an action must be interpreted cautiously because calcium removal would be expected to increase Pi permeability of tight junctions (43) with attendant backflux from the peritubular fluid and reduction of net absorption. In a second study, increasing luminal calcium enhanced Pi absorption (59). Finally, effects of extracellular calcium on proximal Na-Pi cotransport have been described (48). In these latter studies, elevation of calcium increased Pi absorption and vice versa. These findings suggest that calcium directly affects renal Pi absorption. The mechanism responsible for this action is unknown. Calcium could modify renal Pi transport by directly modulating luminal membrane Na-Pi cotransport. Alternatively, calcium could modify the action of PTH on retrieval of apical membrane Na-Pi cotransport.

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Address for reprint requests and other correspondence: P. A. Friedman, Univ. of Pittsburgh School of Medicine, Dept. of Pharmacology, E-1347 Biomedical Science Tower, Pittsburgh, PA 15261 (E-mail: paf10@pitt.edu).

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porter. The actions of calcium on PTH-dependent P;
transport have not been investigated. The calcium-
sensing receptor (CaSR) is expressed on apical mem-
branes of proximal tubules (56). However, the physio-
logical function of proximal tubule CaSRs is unknown.

The second objective of the present studies was to test
the hypothesis that CaSR activation attenuates PTH-
suppressible P;
absorption. Because hypercalcemia alters glomerular ultrafiltration and filtered load, which
in turn might influence renal phosphate reabsorption,
we analyzed the effects of CaSR on phosphate absorp-
tion by single mouse proximal tubules that were mi-
croperfused in vitro.

MATERIALS AND METHODS

Single-tubule microperfusion. The techniques used for
studying phosphate absorption by single in vitro microper-
fused mouse proximal tubules were similar to those
described originally by Burg et al. (13) for isolated rabbit
nephron segments and to those used previously by this labo-

ratory (42) for the study of transport processes in segments
of the mouse nephron. Stated briefly, outhern 25- to 30-day-
old (~25 g) male Institute of Cancer Research white mice
(Harlan, Indianapolis, IN) were killed by cervical dislocation
and rapid exsanguination. All procedures were approved by
Institutional Animal Care and Use Committee protocol
11977. The kidneys were removed, and proximal straight S3
tubules were dissected freehand, without the use of collage-
nase or other enzymatic treatment, from coronal sections of
renal cortex immersed in a HEPES-buffered DMEM media
(Sigma D-2902) containing 6% BSA and maintained at 4°C.

After transfer to a Lucite chamber, tubule segments 0.5–1.0
mm in length were connected to concentric glass pipettes,
and perfusion was initiated by hydrostatic pressure. Tubules
were perfused at average rates of 10–15 nl/min at 37°C, a
rate that is consistent with reported values for single
nephron glomerular filtration rate (GFR) in the mouse (41).
The specific perfusion rates in individual periods varied from
8.7 to 19.3 nl/min in control periods and from 8.5 to 22.3
nl/min in experimental periods. Perfusion rates are indicated
for each set of experiments and did not vary statistically
among groups. Fluid was collected in constant-bore pipettes
under water-saturated mineral oil or tetradecane colored
with phenol red-free DMEM culture media (measured com-
position in mM: 140 NaCl; 5.0 KCl; 1.0 NaH2PO4; 1.8 CaCl2;
0.8 MgSO4; 5.5 d-glucose; 10 HEPES) supplemented with 6%
BSA. The perfusion solution was prepared as an ultrafilterate
(AMicon 52 Ultrafiltration Cell, PM10 membrane; Bedford,
MA) from the bathing solution. 32P and FITC-inulin (Sigma,
150 mg%) were added to the luminal perfusate to measure
phosphate absorption (\(J_{PV}\)) and fluid absorption (\(J_f\)), respec-
tively. All solutions were adjusted to pH 7.4 and 290–300
mosmol/kgH2O and were equilibrated with 100% O2. The
perfusion chamber was bubbled with 100% O2. After three
10-min control periods, 100 nM human PTH(1–34) was
added to the bath and after 20 min three experimental
samples were collected. In other experiments, PTH or the
CaSR agonist GdCl3 was added to the luminal perfusate.
Dopamine was prepared freshly for each experiment in a
stock solution of 200 mM sodium metabisulfite to prevent
oxidation to norepinephrine, which is antiphosphaturic (30).
The stock solution was diluted 1,000-fold to give a final
concentration of 1 μM dopamine in the tubular perfusion
solution. Sodium metabisulfite was added alone during
control periods.

Fluid absorption. Changes in the concentration of FITC-
inulin (Sigma) were used to measure the rate of fluid absorp-
tion (40). The concentration of FITC-inulin in 15-nl samples
of perfusate and collected tubular fluid was determined by a
fluorometric procedure extracted NanoFlo fluorimeter (68)
(World Precision Instruments, Sarasota, FL). The rate of fluid absorption (\(J_f\): nl·min⁻¹·mm⁻²) was calculated accord-
ing to the standard equation

\[ J_f = (\ln [V_i]_L - 1) V/L \]  \hspace{1cm} (1)

where \([V_i]_0\) and \([V_i]_L\) are the concentrations of FITC-inulin in the
collected and perfused fluid samples, respectively. \(V_i\) is
the fluid collection rate, and \(L (\text{mm})\) is the tubule length
measured with an eyepiece micrometer.

Phosphate absorption. The rate of phosphate absorption
was measured with [32P]orthophosphoric acid (ICN). In all
studies, [32P] was present in the luminal perfusate at a con-
centration greater than 20 cpm/nl. Radioactivity of 20-nl
collected samples was measured in duplicate by beta emis-
sion spectrometry (Beckman Coulter LS 6500, Fullerton,
CA). The lumeno-bath absorption of phosphate was calcu-

ated as the difference between the amount perfused and the
amount collected, corrected for fluid absorption according
to the following equation

\[ J_{Piv} = \frac{V_i C_i - V_o C_o}{L} \times \frac{[P O_4]}{C_i} \]  \hspace{1cm} (2)

where \(J_{Piv}\) is the unidirectional lumen-to-bath phosphate flux
(pmol·min⁻¹·mm⁻³), \(V_i\) and \(V_o\) (nl/min) are the fluid perfu-
sion and collection rates, respectively, \(L (\text{mm})\) is the length of
the perfused segment as determined by an eyepiece micro-

meter, \(C_i\) and \(C_o\) (cpm/nl) are the concentration of 33Pi in
the perfusion and collected fluid samples, and \([P O_4]\) is the molar
concentration of P; in the perfusion fluid.

Cell culture. Opossum kidney/epithelial (OK/E) cells were
grown on 25-cm² Falcon dishes (Becton Dickinson, Franklin
Lakes, NJ) in a 50:50 mix of DMEM/F-12 (10–092, Medi-
tech, Herndon, VA) with 15 mM HEPES, 2.5 mM L-glutamine,
and supplemented with 5% FBS (BioWhittaker, Walkers-
ville, MD) and 1% penicillin-streptomycin-neomycin antibi-

otic mixture (Invitrogen Life Technologies, Carlsbad, CA)
in a humidified atmosphere of 95% air-5% CO2 at 37°C. Un-
less stated otherwise, cells were switched to serum-free DMEM/
F12 media 16 h before use.

Phosphate uptake. Confluent cells in 12-well plates were
serum-starved overnight in antibiotic-free media. CaSR acti-
vation was accomplished by augmenting the concentration of
calcium to that indicated, or by the inclusion of NPS 467 to
serum-free media in the presence or absence of hPTH(1–34),
100 nM, for 2 h. Cells were then washed three times with a
Na-containing buffer (in mM: 140 NaCl; 4.8 KCl; 1.2 MgSO4;
0.1 KH2PO4; 10 HEPES) or Na-free buffer, where N-methyl-
-D-glucamine (NMDG) isosmotically replaced Na. Measure-
ment of phosphate uptake was initiated by adding buffer
containing 4 μCi/ml [32P]orthophosphate for 10 min to trip-
licate wells. Uptake is linear over this time frame. Uptake
was stopped by washing three times with ice-cold NMDG
buffer. Cells were extracted overnight with 0.5% Triton
X-100 or 1 N NaOH and then counted by beta-scintillation
spectrometry. Na-dependent P; uptake was calculated by
subtracting uptake in the absence of Na from that in the
presence of Na. Na-independent uptake represents ≤5% of
Na-dependent uptake.
**PTHR1 immunofluorescence.** Mice were anesthetized with pentobarbital sodium (Nembutal; 0.1 ml of a 50 mg/ml solution per 100 g body wt ip) and were perfused through the left ventricle first with PBS (10 mM sodium phosphate buffer containing 0.9% NaCl, pH 7.4) and then with paraformaldehyde-lysine-periodate fixative (PLP) for 5 min as previously described (9). Kidneys were removed and slices were fixed by immersion in PLP at 4°C overnight. Tissues were washed three times in PBS and stored until use in the same buffer containing 0.02% sodium azide.

For immunocytochemical staining, cryostat sections were cut at a thickness of 4 μm. Tissues were cryoprotected by immersion in 0.9 M (30%) sucrose in PBS for at least 1 h before being frozen in liquid nitrogen and sectioned with a Reichert Frigocut cryostat. Sections were picked up on Fisher Superfrost Plus-charged glass slides. Sections were rehydrated in PBS and then treated for 4 min with 0.1% SDS, an antigen retrieval technique, as previously described (10). They were incubated with PBS containing 1% BSA for 15 min and then incubated for 1 h at room temperature with a polyclonal rabbit anti-rat antipeptide antibody (20 μg/ml; PR-630P, rat pep IV) targeted to the extracellular sequence (CTLDDEARLTEEELH) of the PTH1R obtained from Covance (Berkeley, CA). We also localized the CaSR in proximal S3 tubules using a well-characterized CaSR-specific polyclonal antibody [2; (15)] kindly provided by Dr. D. Shoback (University of California, San Francisco). Sections were washed 2 × 5 min in high-salt PBS (PBS containing 2.7% NaCl) and 1 × 5 min in PBS to reduce nonspecific staining, and the secondary donkey anti-rabbit antibody conjugated to CY3 (Jackson Immunologicals) was applied for 1 h at room temperature at a dilution of 1:800. The sections were again washed in high-salt PBS and in PBS before being mounted in Vectashield antifade solution diluted 1:1 in Tris buffer, pH 9.0. Sections were examined using a Nikon Eclipse 800 fluorescence microscope, and images were captured with a Hamamatsu Orca CCD camera and IP Lab acquisition software (Scanalytics).

**Materials.** Human PTH(1–34)NH2 [hPTH(1–34)] was synthesized as described (22). NPS R-467 and S-467 were obtained from NPS Pharmaceuticals, Salt Lake City, UT. All other reagents were purchased from Sigma and were of the highest analytic grade available.

**Statistics.** Data are presented as means ± SE, where n indicates the number of independent experiments. Effects of experimental treatments were assessed by paired comparisons within experiments. Paired results were compared by ANOVA with posttest repeated measures analyzed by the Bonferroni or Tukey procedure (Instat 3; GraphPad, San Diego, CA). The relationship of perfusion rate and JF of JF was analyzed by linear regression analysis (Instat). Differences greater than P = 0.05 were assumed to be significant.

**RESULTS**

**Basolateral addition of PTH inhibits phosphate but not fluid absorption.** Single mouse S3 proximal tubules were dissected and perfused as described in MATERIALS AND METHODS. The perfusion rate was constant between control and experimental periods (14.42 ± 0.42 vs. 13.67 ± 0.52). Under control conditions, JF averaged 2.9 pmol·min⁻¹·mm⁻¹ (Fig. 1A). Lines connect results for 11 independent experiments, where, after adding 100 nM PTH to the basolateral bathing, JF significantly decreased by ~20%. Separate experiments showed no effect of PTH on phosphate backflux from bath to lumen, as reported for rabbit proximal tubules (16). Fluid absorption was measured concurrently in the same experiments. The results shown in Fig. 1B indicate that basolateral PTH had no effect on fluid absorption.

**PTHR1 localization and effects of apical PTH.** Studies were initiated to localize the PTH1R in proximal tubules. Receptor localization was determined using an anti-peptide antibody directed against an epitope in the extracellular domain of the PTH receptor. Figure 2 shows that in mouse S3 proximal tubules, the PTH
rate of phosphate absorption in proximal tubules is

related to the rate of phosphate delivery. Upon addi-
tion of PTH to the basolateral bathing solution, $J_{\text{Pi}}$ decreased and, as expected, phosphate absorption no longer correlated with perfusion rate ($r = 0.19$, not significant). Under the same conditions, there was no statistically significant relationship between perfusion rate and the magnitude of fluid absorption.

**CaSR activation blunts PTH-inhibitable phosphate absorption.** Recent studies show that the CaSR is expressed on BBMs of proximal tubules (56, 57). We confirmed that the CaSR is intensely expressed on BBMs of S3 proximal tubules of the mouse kidney (Fig. 5). However, expression was also detectable along lateral cell membranes and, in some instances, on basal surfaces. The physiological role of the CaSR in proximal tubules is unknown. We theorized that CaSR activation regulates PTH-dependent proximal tubule phosphate absorption, much as it does calcium absorption by cortical ascending limbs (42). Therefore, in the next set of experiments, we tested the effect of CaSR activation on PTH-sensitive phosphate absorption. Gd$^{3+}$, a CaSR agonist, was used to activate the CaSR. Previous studies established that half-maximal CaSR activation occurred at 30 µM Gd$^{3+}$, which corresponds closely to its reported EC$_{50}$ (47, 55). Accordingly, this concentration was used to study the effects of CaSR activation on phosphate absorption. As shown in Fig. 6A, addition of 30 µM Gd$^{3+}$ to the luminal perfusate abolished the inhibitory action of basolateral PTH on phosphate absorption. As a control, Gd$^{3+}$ was added to the serosal bathing solution. In this situation, Gd$^{3+}$ did not block the inhibitory effect of PTH on phosphate transport (Fig. 6B). These findings are consistent with the view that apical membrane calcium receptors regulate PTH-sensitive phosphate absorption by proximal

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**Fig. 2. Immunocytochemical localization of PTH1R in mouse kidney.** A: staining was seen in the apical brush border (arrows) and at the basolateral pole of proximal tubule S3 segments (S3). Considerably stronger staining was present in thick ascending limbs of Henle (TAL), where both apical and basolateral receptors were detectable. This section is from the outer stripe of the outer medulla. B: at higher magnification, apical (*) and basolateral (arrows) staining was often seen in a punctate pattern in proximal tubule S3 segments in the outer stripe of the outer medulla. Bar = 40 µm (A) and 20 µm (B).
Furthermore, Gd³⁺/H11001 had no effect on fluid absorption when added to either lumen or bath (data not shown).

Specificity of CaSR activation for PTH-sensitive phosphate transport. Proximal tubular phosphate absorption is regulated not only by PTH but also by dopamine (4, 19). Addition of 1 μM dopamine to the basolateral solution bathing S3 proximal tubules inhibited J_Pi by 28% (P < 0.01; Fig. 7A). To determine whether CaSR activation is specific for PTH or also affected dopamine-inhibitable phosphate absorption, we used the same protocol described above. Inclusion of 30 μM Gd³⁺ in the luminal perfusate did not affect resting phosphate absorption (2.55 vs. 2.57 pmol·min⁻¹·mm⁻¹). Furthermore, in contrast to its inhibitory effect on PTH-dependent phosphate absorption, Gd³⁺ did not diminish dopamine-sensitive phosphate transport (Fig. 7B).

Characterization of CaSR activation on PTH-sensitive phosphate uptake by OK/E cells. To evaluate further the effect of CaSR activation on phosphate transport, we examined the action of CaSR activation on basal and PTH-inhibitable phosphate uptake by OK/E cells. Figure 8 shows three aspects of phosphate uptake by these cells. First, raising extracellular calcium from 1 to 10 mM had no effect of phosphate uptake. Thus basal phosphate transport is not subject to regulation by the CaSR. Second, at an ambient concentration of 1 mM calcium, PTH inhibited phosphate uptake in a concentration-dependent manner. At 0.1 nM, PTH inhibited phosphate uptake by 10.220.33.2 on June 9, 2017 http://ajprenal.physiology.org/ Downloaded from
duced phosphate uptake by 28% and at 10 nM, PTH uptake was inhibited by 53%. Third, elevation of calcium from 1 to 10 mM blocked the inhibitory effect of PTH on phosphate accumulation. These results show that CaSR activation regulates the PTH-sensitive component of phosphate transport in OK cells.

The specificity of the effects of elevated extracellular calcium for the CaSR was tested by using NPS 467, an organic compound that is a specific calcium receptor-activating ligand (66). The results are shown in Fig. 9. PTH reduced phosphate uptake by 60%. However, in the presence of NPS R-467, the active form of the compound, PTH action was significantly reduced. NPS S-467, the less active optical isomer used as a negative control, did not affect PTH-inhibitable phosphate uptake.

Fig. 6. CaSR activation blocks the inhibitory effect of PTH on $J_{Pi}$. A: basolateral addition of dopamine (10^{-6} M) inhibited $J_{Pi}$ by 28% ($P < 0.01$). B: luminal Gd^{3+} (30 μM) had no inhibitory action on dopamine-sensitive $J_{Pi}$.

Fig. 7. CaSR activation does not interfere with dopamine-inhibitable phosphate absorption. A: basolateral addition of dopamine (10^{-6} M) inhibited $J_{Pi}$ by 28% ($P < 0.01$). B: luminal Gd^{3+} (30 μM) had no inhibitory action on dopamine-sensitive $J_{Pi}$.

Fig. 8. Calcium inhibited PTH-sensitive phosphate uptake by opossum kidney/epithelial (OK/E) cells. Raising extracellular calcium from 1 to 10 mM had no effect on phosphate uptake. Calcium exerted a concentration-dependent inhibition of PTH-sensitive phosphate uptake. At 1 mM calcium, PTH-inhibitable Pi uptake was reduced at 0.1 and at 10 nM PTH. Further elevation of calcium to 10 mM abolished the inhibitory effect of PTH on phosphate uptake at both low- and high-dose PTH.
The present study examined the interactions between PTH and CaSR activation on phosphate absorption. Many factors, including dietary phosphate intake, PTH, and vitamin D, affect phosphate absorption by proximal tubules (20, 23, 35, 44). PTH is the principal short-term regulator of phosphate transport by proximal tubules. Its inhibitory effects are mediated by sequestering the BBM type IIa Na-Pi cotransporter (45). Considerable information, albeit primarily indirect, suggests that changes in extracellular calcium also affect the rate or magnitude of renal phosphate transport. Most studies report that elevating plasma calcium decreases renal Pi excretion (1, 39, 54). The absolute magnitude of PTH-suppressible phosphate transport in these perfused tubule experiments was roughly half that reported in studies of phosphate absorption by rabbit proximal tubules (16). In the rabbit, PTH suppressed phosphate absorption only in proximal straight tubules but not convoluted proximal tubules. In the rabbit proximal tubule, basal phosphate absorption (2.6 pmol·min⁻¹·mm⁻¹) was equivalent to that reported here (2.9 pmol·min⁻¹·mm⁻¹). However, inhibition of phosphate transport by basolateral PTH application in rabbit proximal straight tubules was somewhat greater (28%) than in the mouse (20%). PTH had no inhibitory effect on fluid absorption in rabbit proximal straight tubules when they were perfused with a low-bicarbonate-containing solution. PTH did not alter phosphate backflux in the rabbit (16), dog (26), or rat (26). Similarly, PTH did not alter the rate of phosphate backflux in mouse S3 proximal tubules. Thus qualitatively and quantitatively, the rate of resting and PTH-sensitive proximal tubule phosphate absorption is comparable in the mouse and rabbit. However, the absolute magnitude of PTH-suppressible phosphate transport in these perfused tubule experiments was roughly half that reported in studies of the effect of PTH on phosphate absorption in intact dogs or rats (5, 67). The present findings may explain the difference in the magnitude of the PTH inhibition. In the whole animal studies, PTH action was assessed by comparing tubular phosphate absorption in eunephroathyroid with thyroparathyroidectomized animals. It is now reasonably clear that PTH receptors are expressed on both luminal and basolateral membranes of the proximal tubules (Fig. 2) (2) and that PTH promotes NaPi2 internalization in proximal tubules when they are perfused with either luminal or basolateral solution (64). Because full-length PTH has 84 amino acids, with a nominal size of under 10,000 Da, it will be filtered at the glomerulus and enter the lumen of proximal tubules. Hence, we suggest that in intact animals, the inhibitory action of PTH on proximal phosphate absorption is exerted from both membrane surfaces. Consistent with this suggestion, systemic administration of a parathyroid extract inhibited fractional proximal tubular phosphate reabsorption by some 40% (67). In these instances, the magnitude of the effect of parathyroidectomy or of systemic PTH infusion on proximal phosphate transport compares favorably to that here, where PTH was added to both the luminal perfusate and peritubular bathing solution.

Recent studies using a polyclonal antiseraum raised to an extracellular epitope of the type 1 PTH receptor (PTHR1) described expression at both basolateral and luminal membranes of mouse proximal tubules (2). These results shown in Fig. 2 indicate that the PTH1R is abundantly expressed along the luminal BBM of S3 mouse proximal tubules. The prominent brush-border localization described here contrasts with the work of Amizuka et al. (2), where the PTH1R was primarily expressed at basolateral membranes of proximal tubules. Consistent with an apical membrane PTH1R localization, Traebert et al. (64) reported that when added to luminal or basolateral surfaces of perfused mouse proximal straight tubules, 1 μM PTH(1–34) effectively induced NaPi-IIa sequestration. We sought...
to extend these findings by examining the effect of luminal PTH on phosphate absorption in the same, i.e., S3 proximal tubule segment. We used 100 nM PTH (1–34) because in prior work this was the lowest concentration that elicited maximal cAMP stimulation in mouse proximal tubule cells (21). Normal circulating PTH levels in mice are in the picomolar range (37, 62), whereas the EC50 for the PTH receptor is 1 nM. The reason for this disparity is not entirely understood but may be explained by the possibility that PTH accumulates at the receptor to higher levels than measured in circulation (52). The high in vivo efficacy of PTH may reflect preferential pharmacokinetic and pharmacodynamic properties that result in higher and protracted concentrations of the drug at its target sites in bone and kidney. Alternatively, the extensive amphiphilic helical conformation of the ligand binding site of the PTH1R in vivo may not be represented in the cell culture lines that are used for in vitro characterization of EC50s.

The present results illustrate that PTH reduced phosphate absorption by 30% when added to the luminal perfusate. Combined addition of PTH to both per fusate and basolateral bathing solution further inhibited the magnitude of phosphate absorption to a total of 50%. Over the range of perfusion rates of 8–22 nM/ml, phosphate absorption varied with the rate of tubule perfusion (Fig. 4). This finding indicates that the rate of phosphate absorption by proximal tubules is related to the magnitude of phosphate delivery. Phosphate infusion increased the rate of phosphate reabsorption by proximal convoluted tubules in phosphate-deprived rats (3). In rabbit proximal tubules, however, the rate of phosphate absorption is limited only by the transport capacity of the tubule rather than by the delivery rate (17).

In contrast to proximal tubules and cortical collecting ducts, where the CaSR is expressed on luminal plasma membranes, in TAL the CaSR is primarily located on basolateral plasma membranes (56). In previous studies, we showed that activation of the CaSR in mouse cortical ascending limbs inhibited PTH-stimulated calcium transport (42). CaSR activation in cortical collecting ducts regulates vasopressin-induced water permeability (60). Such an action would diminish the potential for calcium precipitation and renal stone formation. Little is known of the physiological role of the CaSR on proximal tubule solute or fluid absorption. A recent preliminary report suggests that CaSR activation increases 1,25(OH)2vitamin D3 synthesis through activation of the CaSR (8). Because of its important function in regulating renal mineral ion homeostasis, we hypothesized that the proximal CaSR regulates PTH-dependent phosphate absorption. We directly analyzed the effects of CaSR activation on phosphate absorption by single mouse proximal tubules that were micropunched in vitro. For these studies, we used Gd3+ to activate the CaSR. Gd3+ is a potent type I CaSR agonist, with an EC50 of 30–50 μM (47). Using Gd3+ permitted us to avoid millimolar elevations of calcium at one membrane surface or the other, which would directly alter fluid absorption by virtue of its osmotic action. We found that luminal, but not serosal, addition of Gd3+ blocked the inhibitory action of PTH on phosphate absorption. Gd3+ had no effect on basal, i.e., PTH-independent phosphate transport. Previous work by Rouse and Suki (59) demonstrated that calcium did not affect basal phosphate transport in rabbit S3 proximal tubules. These observations are consistent with the predominant apical localization of the CaSR and suggest that the effects of Gd3+ on phosphate absorption were mediated by CaSR activation. Gd3+ had no effect on fluid absorption when added to either lumen or bath. In contrast, a preliminary report described an inhibitory effect of Gd3+ on fluid absorption by proximal tubules but at Gd3+ concentrations (200 μM) considerably greater than employed here (50).

Dopamine also regulates renal phosphate transport (46). The inhibitory effect of dopamine is mediated by D1 and D2 dopamine receptors expressed in the proximal nephron (32). Dopamine inhibits phosphate absorption by rabbit and rat proximal renal tubules (31, 33). In the present investigation, application of dopamine to the basolateral bathing solution inhibited JPi by 28%, somewhat greater than that we observed with basolateral addition of PTH (20%). In contrast, dopamine inhibited phosphate absorption less effectively than PTH in the rat (29). However, in the latter studies, the animals were placed on a low-phosphate diet. Other experimental parameters also differ appreciably from the present report.

More importantly, whereas CaSR activation blocked the inhibitory effect of PTH on phosphate absorption, CaSR activation did not curtail the inhibitory action of dopamine on phosphate transport. Thus CaSR activation specifically inhibits PTH and not dopamine-sensitive phosphate absorption. Again, CaSR activation had no effect on basal phosphate transport.

The inhibitory effects of CaSR activation on PTH-inhibitable phosphate transport were further examined in OK cells, where it is possible to increase calcium without complications due to asymmetric osmotic effects. CaSRs were activated by raising extracellular Ca2+ and by the addition of an unrelated organic compound, NPS R-467, a type II calcimimetic. Elevating extracellular Ca2+ from 1 to 10 mM had no effect on resting phosphate uptake (Fig. 8), similar to the absence of an effect of Gd3+ on basal phosphate transport in the perfused tubule experiments. However, increasing Ca2+ abolished PTH-inhibitable phosphate transport at 0.1 or 10 nM PTH. This finding supports the view that the inhibitory effects observed with Gd3+ are likely attributable to CaSR activation and were not due to blockade of ATP-permeable channels (58), Ca2+-
sensitive K⁺ channels, the nonselective Ca²⁺-permeable cation channel polycystin-2 (25), the Ca²⁺-selective Trp3 channel (49), or other nonselective cation channels (18, 68), or mechanosensitive channels (27). It should be noted that although the solutions containing Gd³⁺ were prepared to a final concentration of 30 μM, the free Gd³⁺ concentration was likely to be substantially lower because trivalent phosphate anions in the extracellular bathing solution avidly bind free Gd³⁺ (14).

The mechanism whereby CaSR activation blocks PTH-inhibitable phosphate absorption has not been examined. Several possible signaling pathways may be involved. Activation of the CaSR most commonly results in G protein-dependent stimulation of phospholipase C with attendant inositol trisphosphate formation and a transient release of Ca²⁺ from intracellular stores (12). Other CaSR signaling pathways, including activation of Gi, phospholipase A₂, phospholipase D, and mitogen-activated protein kinase, have been described but are less well characterized (11, 34).

Mice with targeted inactivation of the CaSR rescued by crossing with PTH-null mice (36) or with Gcm2-null mice (65) do not exhibit statistically significant differences in plasma phosphate. The situation with humans harboring activating CaSR mutations is more complex. Individuals with hypocalcemic hypercalciuria with high serum phosphate have been reported (51, 63). This suggests that CaSR activation and inactivation may have different effects on PTH-sensitive renal phosphate transport. Alternatively, humans and mice may respond differently. It is also quite likely that PTH is not the only peptide hormone regulating proximal nephron phosphate transport. Recent work has clearly identified important roles of FGF23 and FRP4 in renal phosphate absorption (7, 38, 53, 61). Taken together, these findings suggest that CaSR-independent regulation of phosphate homeostasis provides additional levels of defense against significant alterations of extracellular Pi.

The observations reported here show that activation of the apical membrane CaSR specifically attenuates PTH-suppressible Pi absorption in proximal tubules and phosphate uptake by OK/EC cells, a model for the proximal tubule. This effect may represent part of an multitiered hormone and cytokine regulation of renal phosphate transport that involves not only PTH and dopamine but also FGF23 and FRP4 (38).

DISCLOSURES

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