NaPO₄ cotransport type III (PiT1) expression in human embryonic kidney cells and regulation by PTH

ISABELLE FERNANDES,1 RICHARD BÉLIVEAU,2 GERARD FRIEDLANDER,1 AND CAROLINE SILVE1

1Institut National de la Santé et de la Recherche Médicale, Faculté de Médecine Xavier Bichat, 75018 Paris, France; and 2Laboratoire d’Oncologie Moléculaire, Membrane Transport Research Group, Université du Québec à Montréal-Hôpital Stéphanie, Montreal, Quebec, Canada H3C 3P8

Fernandes, Isabelle, Richard Béliveau, Gérard Friedlander, and Caroline Silve. NaPO₄ cotransporter type III (PiT1) expression in human embryonic kidney cells and regulation by PTH. Am. J. Physiol. 277 (Renal Physiol. 46): F543–F551, 1999.—The aim of the present study was to characterize the type(s) of NaPO₄ cotransporter expressed in the human renal cell line HEK-293 and its regulation by parathyroid hormone (PTH) in wild-type cells and in cells transfected by the PTH/PTH-related protein (PTHrP) receptor. The results showed that human embryonic kidney HEK-293 cells expressed NaPO₄ cotransporter type III (PiT1) mRNA and protein. In contrast, type I (NPT1) or II (NPT2) cotransporter mRNA were not expressed. Na⁺-dependent phosphate uptake followed a Michaelis-Menten model (apparent maximal transport rate and affinity constant: 23.32 ± 0.69 nmol PO₄ mg protein⁻¹ 10 min⁻¹ and 0.147 ± 0.014 mM KH₂PO₄, respectively), was stimulated by phosphate deprivation (maximal increase 24.5 ± 0.8%, P < 0.001, after 15 h of phosphate deprivation), and was inhibited by increasing pH (3.6 ± 0.2-fold decrease at pH 8.5, P < 0.0001). It was inhibited in a time- and concentration-dependent fashion by PTH in HEK-293 cells stably transfected by PTHrP receptors but not in parental HEK-293 cells. Maximal inhibition of Na⁺-dependent phosphate transport was observed at 30 min after the addition of 72 nM PTH-(1–34) (31.5 ± 2.4% inhibition, P < 0.01). PTH inhibition of phosphate transport was maintained in phosphate-deprived cells and reversed by both GF109203X (10⁻⁶ M) or staurosporine (5.5 nM), two protein kinase C inhibitors. Na⁺-dependent phosphate uptake was also significantly inhibited by phorbol 12-myristate 13-acetate (20.9 ± 3.9% inhibition, P < 0.001) but not by dibutyryl-cAMP (10⁻⁴ M) or forskolin (50 µM). The physiological role played by type III NaPO₄ cotransporter expression in the overall renal regulation of phosphate homeostasis remains to be established.

human embryonic kidney HEK-293 cells

THREE TYPES OF nonhomologous NaPO₄ cotransporters have recently been characterized at the molecular level (2, 31, 37, 39). Two of these types (types I and II) share 20% homology in amino acid sequences and are expressed almost exclusively in the renal proximal tubules (9, 10). Type I NaPO₄ transporter (designated NPT1, Npt1, and NaPi-1, respectively, in humans, mouse, and rabbit) (39) is expressed in the liver in addition to the kidney (14, 28). Its expression and activity are not regulated by phosphate deprivation or parathyroid hormone (PTH) status, and its role in the regulation of phosphate homeostasis remains unclear (2, 24, 31). Type II cotransporter (designated NaPi-2, NaPi-3 or NPT2, NaPi-4, NaPi-5, NaPi-6 or Npt2, and NaPi-7, respectively, in rat, human, opossum, flounder, vessel, mouse, and rabbit) (2, 31, 37, 39) is a main target for regulation by dietary phosphate content and by PTH, and it ensures the bulk of tubular phosphate reabsorption (2, 24, 31). The key role of NPT2 in regulation of phosphate metabolism has recently been demonstrated by targeted inactivation of the Npt2 gene in mice (Npt2−/− mice) (1) expressing a severe renal phosphate wasting. Type II cotransporter expression has been evidenced in brain (19), osteoclasts (16), lung (29), and very recently small intestine (NaPiIIb) (18) in addition to proximal tubules.

NaPO₄ cotransporters from the third type have been more recently identified and consist of proteins sharing low homology with those of the first two families. These proteins have been originally described as a family of cell surface receptors for gibbon ape leukemia virus (GALV) and murine amphotropic retrovirus (A-MuLV) (22, 23). In contrast to type I and type II NaPO₄ cotransporters, type III NaPO₄ cotransports are ubiquitous in most species (21, 23, 30). The transcripts were prepared from kidney (21, 23, 30) and were found to be expressed throughout the kidney by in situ hybridization (41). High levels of expression are also seen in tissues outside the kidney, including liver, lung, striated muscle, heart, and brain (22, 23). The GALV and A-MuLV receptors are coexpressed on most cells; nevertheless, each virus uses only its own receptor to enter cells (30, 32, 44, 45). These receptors are highly related phosphate symporters (23), and the human homologs are now designated PiT1 (GALV receptor, formerly GLVR1) and PiT2 (A-MuLV receptor, formerly GLVR2 or Ram1) (32, 44). PiT1 is 62% identical in amino acid sequence to PiT2 (22, 30). The PiT1 and PiT2 sequences share <20% identity with the mammalian type I and II NaPO₄ renal cotransporter sequences (22). The predicted amino acid sequences encoded by the PiT cDNAs are characteristic of integral membrane proteins, and hydrophathy analysis suggests the presence of at least 10 potential transmembrane-spanning sequences, with a large hydrophilic domain near the center of each molecule (21, 22). This predicted topological profile differs from that of the renal transporters NPT1 and NPT2, which are modeled to contain between six and eight transmembrane domains (29). The central hydro-
phobic domain of PiTs contains several consensus kinase C and consensus kinase A phosphorylation sites, which may be located intracellularly (21, 22). Thus, these type III NaPO₄ cotransporters appear to represent a family of housekeeping NaPO₄ cotransport systems. Little is known on their role in the overall phosphate homeostasis and on their cellular regulation. They share with type II cotransporter the ability to adapt to extracellular phosphate concentration in vitro, a feature not shared by type I transporters. It is interesting to note that brush border membrane (BBM) vesicles from Npt2−/− mice retains ~30% of the NaPO₄ cotransport activity expressed in BBM from wild-type mice, suggesting that an NaPO₄ cotransporter(s) other than Npt2 is active (1).

Although our knowledge on P_l homeostasis has been greatly enhanced since the molecular characterization of these three types of NaPO₄ cotransporter systems, many aspects regarding the mechanisms involved in phosphate reabsorption by renal tubular cells remain unclear. Key information regarding the mechanisms involved in P_l uptake have been obtained by studies performed in cultured cells. However, most studies were performed before the molecular identification of the different NaPO₄ cotransporter systems. Thus far, only one renal cell line (the opossum kidney OK cells) has been demonstrated to express the type II NaPO₄ cotransporter and PTH/PTH-related protein (PTHrP) receptor, with a conserved PTH regulation of the transporter expression and activity (36). Other renal cell lines such as LLC-PK₁ (3, 6) and Madin-Darby canine kidney (MDCK) (11) have also been used to characterize phosphate uptake, and, for both lines, regulation of NaPO₄ cotransport activity by PTH after stable expression of the PTH/PTHrP receptor has been reported (5, 15, 17). However, the molecular characterization of the NaPO₄ cotransporter type expressed in these cells has not been performed, and the physiological relevance of these studies needs to be documented. Conversely, the human embryonic kidney HEK-293 cell line has mostly been used as a tool to study the properties of transiently or stably transfected proteins, including NaPO₄ cotransporter type II and PTH/PTHrP receptors (20, 35, 38, 42, 43). Compared with other renal cell lines that have also been used to study the properties of transfected proteins, such as MDCK and LLC-PK₁, HEK-293 cells present the advantage of being of human origin rather than of canine (MDCK) or porcine (LLC-PK₁) origin. Furthermore, we have recently demonstrated that PTH stimulates an increase in intracellular calcium in the absence of stimulation of inositol phosphates (InsPs) and cAMP production in wild-type HEK-293 cells but stimulates calcium release through an InsP pathway and induces cAMP production in cells expressing large numbers of PTH/PTHrP receptors after stable transfection of the receptor (20). The aim of the present study was to characterize the type(s) of NaPO₄ cotransporter expressed in HEK-293 cells and its regulation by PTH in wild-type cells and in cells transfected by the PTH/PTHrP receptor.

### MATERIALS AND METHODS

#### Cell Culture

Wild-type HEK-293 cells were purchased from the American Type Culture Collection (Rockville, MD) and were used between passages 40 and 56. Parental HEK-293 cells (referred to as HEK/W) and HEK-293 cells stably transfected with the full-length human PTH/PTHrP receptor cDNA (referred to as HEK/T; see below) were maintained in a humidified atmosphere of 95% air-5% CO₂ at 37°C in DMEM-Ham's F-12 solution (GIBCO BRL, Gaithersburg, MD) supplemented with heat-inactivated FCS and newborn calf serum (5% each), 1 mM glutamine and 100 U/ml penicillin G, and 50 µg/ml of streptomycin. For transport studies, cells were subcultured in 24-well plates coated with collagen I (100 µg/ml; Sigma, St Louis, Mo); 2–5 × 10⁵ cells were seeded in each well in 0.5 ml of culture medium. Cells reached confluence in 4 days, at which time transport experiments were performed. Culture medium was replaced every other day.

#### Transfection

To obtain cells stably expressing the human PTH/PTHrP receptor, HEK/W cells were cotransfected with a pSV2 neo-plasmid and 3- to 20-fold excess of a full-length human PTH/PTHrP receptor cDNA inserted in pcDNA 1 plasmid as described (20). The clone selected for this study (HEK/T) stably expressed ~3.5 × 10⁴ receptors/cell and responded to PTH by an increase in cAMP and InsP productions and by a liberation of calcium from intracellular compartments via a phospholipase C-sensitive pathway (20).

#### Extraction of Cellular RNA

Total RNA was extracted from confluent cells grown in 56-cm² culture dishes according to the method of Chomczynski and Sacchi (7) and stored at −80°C. RNA concentration was determined by absorbance at 260 nm, and RNA integrity was evaluated by electrophoresis on 1% agarose gels containing ethidium bromide.

#### Reverse Transcription and Amplification by PCR of cDNA Coding for Different NaPO₄ Cotransporter

cDNA was synthesized by reverse transcription of 5 µg of total RNA prepared from confluent HEK/W or HEK/T cells with the use of 400 U of Moloney murine leukemia virus RT (GIBCO BRL) and random hexamers as primers (1 µg of pDN6; Pharmacia Biotech, St. Quentin Yvelines, France) in a final volume of 50 µl with the use of 1 U of Taq DNA polymerase (GIBCO BRL) in the presence of 2.5 mM MgCl₂, 0.25 mM each of deoxynucleotides (dNTP), and 0.5 pmol of each primer. The sequence of the oligonucleotide primers used for amplification for each NaPO₄ cotransporter is described in Table 1 and was based on the human cDNA sequence for NPT1, NPT2, and PIT1 reported by Chong et al. (8), Magagnin et al. (29), and O’Hara et al. (32), respectively. Cycling parameters were as follows: 94°C/30 s, 55°C/30 s, and 72°C/30 s, for 35 cycles followed by 10 min at 72°C. Control PCR runs included amplification of samples 1) treated by deoxyribonuclease I (1 U/µg RNA; Pharmacia Biotech), 2) without reverse transcription, and 3) without template.

#### Restriction-Enzyme Analysis of PCR Product

The 215-bp PCR product obtained with the use of primers specific for PIT1 was digested by Msp I (GIBCO BRL) under conditions suggested by the manufacturer, and digestion...
products were visualized by ultraviolet illumination after electrophoresis on a 1% agarose gel containing 0.5 µg/ml of ethidium bromide. Digestion of the PCR product generated two fragments (185 and 30 bp).

Crude Membrane Extraction and Western Blotting

Confluent HEK/W and HEK/T cells in 60-cm² plates were first washed twice with DMEM-Ham's F-12 and lysed with 3 ml of buffer A (5 mM Tris·HCl, pH 7.4, and 1 mM EDTA) containing 0.2 mM phenylmethylsulfonyl fluoride (a protease inhibitor) (Sigma, St. Quentin Fallavier, France). The plates were scraped with a disposable cell scraper, and lysates were removed and homogenized in a Dounce homogenizer (piston B) by 15 up-down strokes and centrifuged at 1,000 rpm for 10 min at 4°C. The supernatant was recovered and centrifuged at 20,000 g for 30 min at 4°C. The pellet of crude membranes was solubilized in 30 µl of buffer A and preserved at −80°C.

SDS-PAGE was carried out according to the procedure of Laemmli (26). A mass of 20 µg of crude membranes was solubilized in 95 mM Tris·HCl (pH 6.8), 20% glycerol, 2% SDS, and 143 mM 2-mercaptoethanol in a total volume of 20 µl. Proteins were separated by SDS-PAGE (10% gel), and gel was electrotransferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA). Blots were blocked with 50 mM Tris·HCl, pH 7.5, 150 mM NaCl, and 0.2% Tween 20 for 1 h and then incubated overnight at 4°C with rabbit anti-PiT1 antibodies, raised against a portion of human PiT1 NaPO₄ cotransporter (Sigma, St. Quentin Fallavier, France). The antibody complexes were visualized with the use of enhanced luminosol-based reagent (ECL) reagent (Amersham, Les Ullis, France) according to the manufacturer's instructions. Exposure to ECL film (Amersham) and 0.1 mM KH₂PO₄, except that Na⁺-independent phosphate uptake was measured in the presence of 137 mM N-methylglucamine-hydrochloride and not choline chloride. Na⁺-dependent Pi transport (nmol·mg protein⁻¹·10 min⁻¹) is the difference between total Pi transport and Na⁺-independent Pi transport. [³²P]Phosphate uptake without Na⁺ was calculated by 10.220.33.4 on June 9, 2017 http://altprenal.physiology.org/ Downloaded from

### Table 1. Sequence and position of primers used to amplify different NaPO₄ cotransporters

<table>
<thead>
<tr>
<th>NaPO₄</th>
<th>Sequence</th>
<th>Size of Amplified Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPT1</td>
<td>5' AAC GAG GCC GAC TCA CTT CTA TGA 3'</td>
<td>237 bp</td>
</tr>
<tr>
<td>NPT2</td>
<td>5' GCT GGA CCA GGG AGG ATG TGA TGT 3'</td>
<td>302 bp</td>
</tr>
<tr>
<td>PIT1</td>
<td>5' GCC ATG TTC GAA AAG AA 3'</td>
<td>215 bp</td>
</tr>
</tbody>
</table>

Primers in bold letters are complementary to the (+) strand. NPT1, type I NaPO₄ cotransporter; NPT2, type II NaPO₄ cotransporter; PIT1, type III NaPO₄ cotransporter.

Alkaline Uptake Measurements

Na⁺-dependent transport of alanine was measured with the use of 5 µCi/ml of l-[³H]alanine (Amersham), as previously described (11).

Kinetics of Na⁺-Dependent Phosphate and Alanine Transports

The uptake of phosphate was measured for 10 min in transport media (pH 7.4) containing different phosphate concentrations (1.25, 25, 50, 100, 250, 500, and 1,000 µM) at 37°C. The uptake of alanine was measured for 5 min in the media transport containing different alanine concentrations (0.1, 0.25, 0.5, 1, 2.5, and 5 mM) at 37°C. Apparent affinity constant (Kₘ) and maximal transport rate (Vₘₐₓ) were calculated by nonlinear curve fitting, assuming Michaelis-Menten kinetics.

Expression of Data and Statistical Analysis

Unless otherwise stated, results are expressed as means ± SE. All transport experiments were performed in triplicate. For statistical analyses performed, see figure legends. P < 0.01 was considered significant. Similar results were obtained in sham-transfected and wild-type HEK-293 cells.

### RESULTS

Identification of NaPO₄ Cotransporters Expressed in HEK Cells by RT-PCR

When RT-PCR was performed from RNA prepared from HEK/T and HEK/W cells with the use of oligo-
Characterization of Phosphate Uptake in HEK Cells

Time course. The time course of Pi uptake in the presence of NaCl or N-methyl-β-glucamine illustrates the Na⁺ requirement for Pi entry in HEK cells (Fig. 3). Phosphate uptake by HEK cells measured in medium containing Na⁺ increased linearly with incubation time for the first 20 min at 37°C and was maximal at ~30 min, at which time phosphate uptake was 26.9 ± 0.9 nmol Pi/mg protein (3 independent experiments) (Fig. 3). When Na⁺ was replaced with equimolar N-methyl-β-glucamine, phosphate uptake did not significantly change as a function of time and was ~7% of that measured in the presence of Na⁺ (0.4 ± 0.04 nmol Pi/mg protein). All other measurements of phosphate uptake were performed under linear uptake rates of 10 min.

Kinetic analyses. To determine the apparent kinetic parameters for phosphate, phosphate uptake was measured as a function of extracellular phosphate concentration. Na⁺-dependent phosphate uptake was a saturable process and followed a Michaelis-Menten model. Analysis of the kinetics parameters, calculated by nonlinear curve fitting, indicated similar values for apparent Vₘₐₓ and Kᵦₚ in HEK/W and HEK/T cells (Fig. 4). Dependence on extracellular pH. Na⁺-dependent phosphate uptake was markedly decreased by raising external pH from 7.4 to 8 and further declined at pH 8.5 (2.3 ± 0.4- and 3.6 ± 0.2-fold decreases, respectively, at pH 8 and pH 8.5 compared with the uptake measured at pH 7.4; P < 0.0001, n = 7; Fig. 5). Increasing external pH from 7.4 to 8.5 had no significant effect on Na⁺-independent phosphate uptake (Fig. 5). Similar results were obtained in HEK/W and HEK/T cells (results are shown for HEK/W).

Modulation by phosphate deprivation. Incubation of HEK cells in phosphate-deprived culture medium increased phosphate uptake in a time-dependent manner.
compared with that in cells incubated with medium containing 1.2 mM phosphate for the same time (Fig. 6). Phosphate uptake significantly increased 1 h after phosphate deprivation with a maximum uptake at 15 h after phosphate deprivation (14.6 ± 0.2 and 24.5 ± 0.8% increase, respectively, compared with that measured in phosphate-containing medium; \( P < 0.01 \) and \( P < 0.001, n = 3 \)). Na\(^+\)-independent phosphate uptake was not significantly changed by phosphate deprivation of the culture medium (data not shown). Similar results were obtained in HEK/W cells (results are shown for HEK/W).

Fig. 6. Effect of phosphate deprivation on Na\(^+\)-dependent phosphate uptake by HEK/W cells. Cells were incubated for indicated times in DMEM-Ham's F-12 culture medium containing 1.2 mmol phosphate/l (○) or no phosphate (●) and uptake was measured for 10 min as described in MATERIALS AND METHODS. Values are means ± SE of 3 experiments performed in triplicate. Statistical analysis was performed with the use of 2-way ANOVA. \(^{***} P < 0.0001\), comparison of cells subjected to phosphate deprivation vs. those not subjected. Similar results were obtained in HEK/T cells.

PTH Regulation of Phosphate Transport in HEK Cells

PTH inhibited Na\(^+\)-dependent phosphate uptake in a time- and concentration-dependent fashion in HEK/T cells. PTH inhibition of phosphate uptake was rapid and transient. Maximal inhibition of Na\(^+\)-dependent phosphate was observed at 30 min after the addition of 72 nM bPTH-(1—34) (31.5 ± 2.4% inhibition, \( P < 0.01 \)), after which time Na\(^+\)-dependent phosphate uptake increased progressively to reach values not significantly different from those observed in control cells after 1 h (Fig. 7). PTH inhibition of phosphate uptake was maintained in phosphate-deprived cells; the time...
course of the inhibition was similar to that seen in non-phosphate-deprived cells (Fig. 7). The PTH inhibitory effect, when studied under these conditions, is higher in absolute terms in Pi-adapted cells compared with non-Pi-adapted cells (5.2 ± 0.25 and 4.0 ± 0.31 nmol Pi/mg protein 1-10 min -1 inhibition, respectively, in Pi-adapted cells and in non-Pi-adapted cells; P < 0.01). As depicted in Fig. 8, PTH exerted its inhibition on Pi uptake in a concentration-dependent manner, and Na+-dependent phosphate uptake was significantly inhibited by 7.2 nM PTH (18.6 ± 3.5% inhibition; P < 0.01, n = 3); maximal inhibition was obtained by 240 nM PTH (Fig. 8). PTH inhibition of phosphate uptake was related to a decrease in Vmax of the transporter PTH (21.3 ± 0.9% inhibition; P < 0.001, n = 4; Fig. 9). This effect of PTH was specific for phosphate transport, as PTH had no effect on Na+-dependent alanine transport (Fig. 10). PTH had no effect on phosphate uptake in HEK/W cells (Fig. 8).

Pathways Involved in PTH-Induced Inhibition of Phosphate Uptake

In an attempt to characterize the cellular pathways involved in PTH-induced inhibition of Na+-dependent phosphate uptake, the effects of exogenous db-cAMP, forskolin, and PMA on Na+-dependent phosphate uptake were first characterized. Previous studies from our group (20) and others (35, 38) have demonstrated that PTH stimulates cAMP and InsP production in HEK/T cells. Results showed that neither db-cAMP (10-4 M) nor forskolin (50 µM) inhibited Na+-dependent phosphate uptake (Fig. 11). In contrast, Na+-dependent phosphate uptake was significantly inhibited by PMA (20.9 ± 3.9% inhibition; P < 0.001, n = 3). The effects of PTH in the presence of GF109203X (10-6 M) or staurosporine (5.5 nM) [two inhibitors of protein kinase C (PKC)] were then studied. PTH inhibition of phosphate transport was reversed by both PKC inhibitors (Fig. 11). Neither compound alone had a significant effect on Na+-dependent phosphate uptake. PTH effect was studied in two different clones of PTH/PTHrP receptor-transfected cells (expressing approximately the same number of receptors), and similar results were obtained in both clones.
The absence of NaPO4 cotransporter type I is not modified (2). A slight decrease in type IIb-associated NaPO4 cotransport activity was recently described (18). However, this pH dependency was small, and, furthermore, NaPO4 cotransporter type IIb is not expressed in the kidney. These results strongly suggest that HEK-293 cells do not express type I or II NaPO4 cotransporter, although it cannot be ruled out that they express an as-yet-unidentified NaPO4 cotransporter. The absence of NaPO4 cotransporter type I has recently been reported in HEK-293 cells (42), in agreement with our data. In the same study, NaPO4 cotransport type II expression was reported. However, an important decrease in “native” NaPO4 cotransport activity was observed as a function of an increase in extracellular pH, which is difficult to reconcile with the pH dependency described for type II cotransport activity. The observed pH dependency of NaPO4 cotransport activity would be compatible with the presence of NaPO4 cotransport type III, although the molecular expression of NaPO4 cotransport type III was not assessed.

Having characterized the type of NaPO4 cotransport expressed in HEK-293 cells, and, in an effort to shed some light on and integrate various data from the literature describing regulation of NaPO4 cotransport activity by PTH, we next studied the regulation of NaPO4 activity by PTH in HEK/W cells and in HEK/T cells. A previous study from our laboratory (20) demonstrated that PTH induces an increase in intracellular calcium concentration in the absence of stimulation of InsP and cAMP production in HEK/W cells but stimulates calcium release associated with an increase in InsP pathway production (thus most likely stimulating PKC activation) and induces cAMP production in HEK/T cells. In the present study, it is demonstrated that PTH stimulation of HEK/W cells does not regulate NaPO4 cotransport activity. In contrast, PTH stimulation of HEK/T cells leads to a time- and concentration-dependent inhibition of NaPO4 cotransport activity associated with a decrease in the Vmax of the cotransport, without change in affinity for phosphate. This effect of PTH was specific for phosphate, as alanine uptake was not modified by PTH treatment of the cells. The results obtained in HEK/W and HEK/T cells are compatible with the possibility that PTH inhibits NaPO4 cotransport activity in HEK/T cells via PKC-activated pathways. This hypothesis is compatible with the presence of potential phosphorylation sites by PKC in the cotransporter type III sequence (21, 32). In support of that possibility, NaPO4 cotransport was also inhibited by PKC activation, but not by protein kinase A (PKA), and the addition of two PKC inhibitors reversed PTH inhibition. Because HEK-293 cells express Na-Pi type III (PiT1) and not Na-Pi types I and II, it seems reasonable to assume that the NaPO4 cotransport activity shown in the present study to be inhibited by PTH and PKC activation was mediated by Na-Pi, type III. Recently, it has been shown that PTH inhibition of Na-Pi, type II cotransport activity is associated with the stimulation of Na-Pi, type II transporter endocytosis, followed by degradation (34). It is unlikely that PTH inhibition of Na-Pi, type III cotransport activity in HEK/T cells involves endocytosis, because 1) the effect of PTH in HEK/T cells is transient, and 2) Na-Pi cotransport types II and III are expressed at the BBM and basolateral membrane, respectively, of cells (unpublished results). These membranes have different structural properties, suggesting that the interaction between Na-Pi cotransport types II and III and the cytoskeleton is different.

In a recent study by Lederer et al. (27), it was demonstrated that PTH stimulation of OK cells leads to...
a decrease of Na-Pi cotransport activity associated with a decrease of type II expression at the membrane via PKA activation, whereas PTH activation of PKC induced a decrease in Na-Pi cotransport activity without a change in expression in type II. It has been demonstrated that OK cells express type III cotransporter in addition to that of type II (4). Because the activity of these transporters cannot be readily differentiated, it is possible that part of the PTH effect described resulted from PTH regulation of both type III and type II cotransport activities. An absence of regulation by PTH of Na-Pi type III cotransporter protein (studied by Western blot) and activity has been reported in OK cells (4) and in mouse distal convoluted tubule (MDCT) cells (40), respectively. However, in these studies, the PTH effect was studied after 2 h (OK cells) and 4 h (MDCT cells) of incubation with PTH, at which time we did not observe a PTH inhibitory effect.

We found that PTH inhibits NaPO4 cotransport activity in HEK-293 cells, in contrast to the effect of PTH in other cell lines, namely, MDCK and LLC-PK1 (5, 15, 17). When these latter cell lines were transfected by PTH/PTHrP receptors, it was found that PTH stimulates NaPO4 activity. Although the NaPO4 cotransporter expressed by these cell lines has not been characterized, it is tempting to speculate that it is NaPO4 cotransporter type III. Similarly, it has been reported that PTH stimulates NaPO4 cotransport activity in osteoblast-like cells that do not express type I or II NaPO4 cotransporter but do express type III cotransporter (33). Thus these data would suggest that regulation of type III NaPO4 cotransporter is cell specific. However, it is interesting to point out that in both cases (PTH inhibition or stimulation of NaPO4 cotransport activity), the PTH effect appears to be mediated by an activation of PKC, not PKA. The regulation of NaPO4 cotransport activity observed in HEK-293 cells is similar to that described in primary cultures of renal proximal tubular cells (13, 25). Interestingly, these cells at confluence do not express NaPO4 cotransporter mRNA type II or I but do express type III (unpublished results), and NaPO4 cotransport activity in these cells is rapidly inhibited by PKC activity (13).

In conclusion, the renal human cell line HEK-293 expresses only type III NaPO4 cotransporter, but not type I or II. Regulation of this transporter activity by PTH in these HEK/T cells leads to an inhibition of the cotransporter that most likely involves PKC activation but not an increase in intracellular calcium or calcium concentrations. These results underline the possibility that in cell lines expressing type II cotransporter, but also type III, such as OK cells, the observed PTH effect on NaPO4 cotransport activity results from a regulation of different NaPO4 cotransport activities. These findings may explain some discrepancies observed in studies describing the mechanisms involved in PTH regulation of NaPO4 cotransporter activity in different cell lines, including subclones of OK cells. The physiological role played by type III NaPO4 cotransport in the overall renal regulation of phosphate homeostasis remains to be established; keep in mind that 1) mRNA expression has been evidenced throughout the kidney (41), and 2) protein expression has been demonstrated at the basolateral and not apical membranes prepared from rat renal cortices (unpublished results). Because type III NaPO4 cotransport is ubiquitously expressed and is located in the basolateral membrane of proximal tubules, it is unlikely that its main role is to regulate phosphate reabsorption by the renal proximal tubule. If the PTH regulation evidenced is taking place in vivo, which remains to be determined, one could speculate that it participates in the cellular phosphate metabolism.

We are grateful to Maurice Bichara for a careful reading of the manuscript.

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, and the Association pour l’Utilisation du Rein Artificiel.

Isabelle Fernandes is a recipient of a fellowship from the Ministère de la Recherche et de la Technologie.

Address for reprint requests and other correspondence: C. Silve, INSERM U 426, Faculté de Médecine Xavier Bichat, 16 rue Henri-Huchard, BP 416, 75870 Paris cedex 18, France (E-mail: silve@bichat.inserm.fr).

Received 11 January 1999; accepted in final form 11 June 1999.

REFERENCES


