Regulation of mOAT-mediated Organic Anion Transport by Okadaic Acid and Protein Kinase C in LLC-PK₁ Cells*

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Organic anion transporters in the kidney proximal tubule play an essential role in eliminating a wide range of organic anions including endogenous compounds, xenobiotics, and their metabolites, thereby preventing their potentially toxic effects within the body. We have previously cloned a cDNA encoding an organic anion transporter from mouse kidney (mOAT) (Lopez-Nieto, C. E., You, G., Bush, K. T., Barros, E. J. G., Beier, D. R., and Nigam, S. K. (1997) J. Biol. Chem. 272, 6471–6478; Kuze, K., Graves, P., Leahey, A., Wilson, P., Stuhlmann, H., and You, G. (1999) J. Biol. Chem. 274, 1519–1524). In the present study, we assessed the potential for regulation of this transporter by heterologous expression of mOAT in the pig proximal tubule-like cell line, LLC-PK₁. We report here that both protein phosphatase (PP1/PP2A) inhibitor, okadaic acid, and protein kinase C (PKC) activators down-regulate mOAT-mediated transport of para-aminohippuric acid (PAH), a prototypic organic anion, in a time- and concentration-dependent manner. However, their mechanisms of action for this down-regulation are distinct. Okadaic acid modulated PAH transport, at least in part, through phosphorylation/dephosphorylation of mOAT; phosphoamino acid analysis indicated this phosphorylation occurs on serine. In contrast, PKC activation induced a decrease in the maximum transport velocity (Vₘₐₓ) of PAH transport without direct phosphorylation of the transporter protein. Together these results provide the first demonstration that regulation of organic anion transport by mOAT is likely to be tightly controlled directly and indirectly by phosphatase PP1/PP2A and PKC. Our results also suggest that kinases other than PKC are involved in this process.

Renal organic anion transport plays a vital role in the elimination of a wide variety of potentially toxic and negatively charged waste products of metabolism, drugs, environmental pollutants, and their metabolites from the body. The transport mechanisms responsible for this elimination have been extensively studied (3–5). Based on these studies, it has been suggested that the transport of organic anions is a complex process involving distinctly different proteins at the apical and basolateral membranes of the proximal tubule cells. Organic anions are transported across the basolateral membrane into the cell in exchange for intracellular dicarboxylates, which are subsequently returned into the cell via a sodium-dependent dicarboxylate transporter. Once inside the cell, organic anions are subject to intracellular binding and sequestration within vesicular structures. Finally, luminal exit is thought to occur by anion exchange and/or facilitated diffusion (3–5).

We (1, 2) and others (6–11) have recently cloned the organic anion transporter cDNA from kidneys of multiple species. Using computer modeling based on hydropathy analysis, the predicted proteins share several common features, including 12 putative membrane-spanning segments, a cluster of potential glycosylation sites located in the first extracellular loop between transmembrane domains 1 and 2, and multiple presumptive phosphorylation sites. Recent progress from our laboratory on the study of structure/function relationships in mOAT using heterologous expression systems has shown that histidine residues are important for the transport function, and glycosylation is necessary for the targeting of mOAT to the plasma membrane (2).

The presence of potential phosphorylation sites on these proteins suggests that they may be subject to phosphorylation-induced functional regulation, and several studies have indicated that transport of organic anions is affected by PKC activators (9, 12–15). In this report we provide the first evidence that the phosphatase inhibitor, okadaic acid, and PKC activators both modulate mOAT-mediated transport function. However, their mechanisms of action are distinct. Okadaic acid regulates the transport, at least in part, through the phosphorylation of serine residue(s) of mOAT, whereas PKC modulates the transport function by changing the maximum transport velocity without direct phosphorylation of the transporter protein. We also suggest that kinases other than PKC are involved in the regulation of organic anion transport.

EXPERIMENTAL PROCEDURES

Materials—[32P]Orthophosphate was obtained from ICN. Protease inhibitor complex was from Roche Molecular Biochemicals. Protein A-agarose beads were from Life Technologies, Inc. Phosphoamino acid standards (Ser(P), Thr(P), and Tyr(P)) were from Sigma. Phorbol 12-myristate 13-acetate (PMA), phorbol 12,13-dibutyrate (PDBu), 4α-phorbol 12,13-didecanoate (4α-PDD), 1-octeyl-2-acetly-sn-glycerol (OAG), and indolactam were also purchased from Sigma.

Construction of myc-tagged mOAT cDNA—Full-length cDNA encoding mOAT with the c-myc epitope tag, was subcloned into the mamma-

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lian expression vector pcDNA3.1(2). The epitope-tagged constructs encoded a fusion protein consisting of full-length mOAT with 10 amino acids of the human c-Myc epitope (EQKLISEEDL, nucleotide sequence GAACAAAAGCTGATTTCTGAAGAAGACCTG) at the carboxyl terminus. Tagged mOAT cDNA was synthesized by polymerase chain reaction amplification. The polymerase chain reaction product was subcloned into the plasmid pcDNA3.1(2) at XbaI and HindIII sites. The nucleotide sequence was confirmed by the dideoxy chain-termination sequencing method (16).

**Generation of LLC-PK<sub>1</sub> Cells Expressing mOAT and mOAT-myc**—LLC-PK<sub>1</sub> cells purchased from the American Type Culture Collection were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, in a 5% CO<sub>2</sub> atmosphere at 37°C. LLC-PK1 cells were seeded at 1 × 10<sup>5</sup>/100-mm dish/10 ml of complete medium 24 h before transfection. For transfection a DNA-calcium phosphate precipitate (1 ml) formed with 20 μg in total of plasmid DNA was added and the cells were incubated at 37 °C for 24 h, washed, and incubated further. After 14–20 days of selection in medium containing 2 mg/ml Geneticin (G418; Life Technologies, Inc.) following the manufacturer’s instruction, resistant colonies were replated to 96 wells for cloning, expanded, and used for analyzing positive clones.

**Cell Culture**—LLC-PK<sub>1</sub> cells expressing mOAT or mOAT-myc established above were maintained in complete medium consisting of DMEM supplemented with 10% fetal bovine serum. Monolayers were grown under an atmosphere of 5% CO<sub>2</sub>, 95% air at 37°C, and were subcultured every 2–3 days using 0.02% EDTA and 0.05% trypsin. For the transport assay, confluent cell monolayers were cultured in Transwell chambers (0.4-μm pores) in 12-well plates (Costar, Cambridge, MA). The volume of medium inside and outside the chambers was 0.5 and 1.5 ml, respectively.

**Transport Measurements**—Uptake of [14C]PAH was initiated by adding uptake solution (PBS, pH 7, containing 5 mM glucose and [14C]PAH) to either the basal or apical side of the monolayers. At times as indicated in the figure legends, the uptake was stopped by rapidly washing the cells with ice-cold PBS. The cells were then solubilized in 0.2N NaOH and aliquoted for liquid scintillation counting. Uptake count was

**FIG. 1. Generation of LLC-PK<sub>1</sub> cells expressing mOAT and mOAT-myc.**

*a*, Northern blot analysis of total RNA isolated from LLC-PK<sub>1</sub> cells expressing pcDNA3.1(−) alone (control, lane 1), pcDNA3.1(−)-mOAT (lane 2), and pcDNA3.1(−)-mOAT-myc (lane 3). *b*, Western blot analysis with crude membranes from cells expressing pcDNA3.1(−) alone (control, lane 1) and pcDNA3.1(−)-mOAT-myc (lane 2). The blot was probed with anti-myc antibody and horseradish peroxidase-labeled secondary antibody and visualized by ECL system.

**FIG. 2. PAH uptake into cells expressing mOAT and mOAT-myc.**

*a*, cells were seeded on transwells, and directional uptake of PAH (20 μM, 20 min) from either apical sides (dotted columns) or basal sides (solid columns) were performed. *b*, time course of basolateral uptake of PAH (20 μM). Values are mean ± S.E. (*n* = 3).

**FIG. 3. Immunolocalization of mOAT-myc in LLC-PK<sub>1</sub> monolayers by confocal laser-scanning microscopy.** Indirect immunofluorescence (fluorescein isothiocyanate) picture with anti-myc antibody. *a* and *b* represent top view of the cell monolayer (seeded on plastic wells), in which *a* corresponds to LLC-PK<sub>1</sub> transfected with pcDNA3.1(−)-mOAT-myc and *b* corresponds to LLC-PK<sub>1</sub> transfected with pcDNA3.1(−) alone. *c*, *d*, and *e* represent optical sections parallel to the plane of the cell layer (seeded on transwells), in which *c* corresponds to apical plane, *d* corresponds to lateral plane, and *e* corresponds to basal plane. *f* represents optical section perpendicular to the plane of the cell layer. Arrows indicate the positions of apical (ap) and basal (bl) surfaces. The bar is 10 μm.
myc (stably transfected with pcDNA3.1(2) was added, and the cells were lysed. RIPA buffer consisted of 10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, pH 7.4, with the protease inhibitor complex, and phosphatase inhibitors. The lysate was centrifuged at 13,000 g for 10 min at 4 °C, discarding the pellet, and the supernatant was incubated with 3 l of 2 mCi/ml [32P]orthophosphate in phosphate-free DMEM for 1 h at 37 °C and incubated with 1 μM okadaic acid (OA) for various times. RIPA extraction, immunoprecipitation, SDS-PAGE, and autoradiography were performed as described under "Experimental Procedures." a, representative autoradiogram of labeling results. b, quantitation of mOAT labeling. c, 1-min basolateral uptake of PAH (20 μM) into the cells treated with 1 μM okadaic acid for indicated time periods. Values are mean ± S.E. (n = 3).

**FIG. 4.** In vivo phosphorylation of mOAT in LLC-PK₁ cells. Cells stably transfected with pcDNA3.1(−) (lane 1) or pcDNA3.1(−)-mOAT-myc (lane 2) were labeled with [32P]orthophosphate and incubated with okadaic acid. Cell lysates were immunoprecipitated with anti-myc antibody, electrophoresed, and autoradiographed. The arrow indicates phosphorylated protein.

standardized by the amount of protein in each well. Values were mean ± S.E. (n = 3).

**Northern Blot Analysis—**Five μg of total RNA prepared from stable transfecnt clones was electrophoresed on a 1% agarose/formaldehyde gel and transferred to nitrocellulose. The membrane was hybridized at 42 °C overnight in hybridization solution with full-length mOAT cDNA labeled with [32P]dCTP by random primer labeling, followed by purification using a Sephadex G-50 spin-column (Amersham Pharmacia Biotech). The filter was washed in 0.2x SSC, 0.1% SDS at 65 °C and subjected to autoradiography.

**Cell Membrane Isolation—**Cells grown on a 100-mm dish were homogenized in isolation solution (250 mM sucrose, 10 mM triethanolamine, pH 7.6) containing complete protease inhibitor mixture (Roche Molecular Biochemicals). Crude membranes were obtained by centrifuging at 1000 × g for 10 min at 4 °C, discarding the pellet, and centrifuging the supernatant at 17,000 × g for 20 min. The resulting pellet was resuspended in isolation solution plus protease inhibitors.

**Electrophoresis and Immunoblotting—**Protein samples were loaded (20 μg/lane) on 7.5% SDS-PAGE minigels and electrophoresed using a mini cell (Bio-Rad). Proteins were transferred to PVDF membranes in an electroelution cell (Bio-Rad). The blots were blocked for 1 h with 5% nonfat dry milk in PBS-Tween (80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% Tween 20, pH 7.5), washed, and incubated for 1 h at room temperature with anti-Myc monoclonal antibody (1:400). The membranes were washed, incubated with goat anti-mouse IgG conjugated to horseradish peroxidase (1:5000), and signals were detected by enhanced chemiluminescence (ECL; Amersham).

**Confocal Laser Scanning Microscopy—**Cells were examined using a Leica TCS-SP (UV) confocal laser scanning microscope (Heidelberg, Germany) equipped with a 100 × 1.4 numeric aperture objective lens. The pinhole size was adjusted such that resultant "optical sections" were approximately 0.5 μm in thickness. Images were collected in the xy plane (from serial optical sections generated at 0.5-μm intervals from the top to the bottom of the cells) or in the xz plane.

The sensitivity of the photomultiplier detectors was set such that the intensity levels of the output signal in the plane of maximum fluorescence intensity were distributed in a linear fashion (via a glow over/under lookup table) over 256 gray levels (with the dimmest pixel, black = 0 and the brightest pixel, white = 255). For control studies (in which the primary antibody was omitted), the detector settings were maintained at the values used in the studies in which primary antibody was present.

**Metabolic Labeling and Immunoprecipitation—**Stably transfected LLC-PK₁ cells were seeded on six-well plates. (approximately 2 × 10⁶/well). The cells were washed once with phosphate-free DMEM without serum or antibiotics and incubated with 2 μl of this medium at 37 °C for 1 h. The cells were labeled by addition of 25 μl of 2 μCi/ml [32P]orthophosphate (50 μCi/well/ml) and incubated for 3 h at 37 °C. Okadaic acid or PKC activators at various concentrations (see figure legend) were added to the medium, and the incubation was continued at 37 °C. The treated cells were washed once with ice-cold PBS 400 μl of ice-cold RIPA buffer containing protease and phosphatase inhibitors was added, and the cells were lysed. RIPA buffer consisted of 10 mM Tris, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, pH 7.4, with the protease inhibitor complex, and phosphatase inhibitors: 10 mM sodium fluoride and 1 μM okadaic acid. The lysate was centrifuged at 13,000 × g for 30 min at 4 °C. The supernatant was precleared with 100 μl of protein A-agarose beads for 1 h with shaking at 4 °C. The lysate was centrifuged at 200 × g for 1 min, and the supernatant was incubated with 5 μl of anti-myc monoclonal antibody at 4 °C overnight, with end-over-end continuous mixing. Then, 20 μl of protein A-agarose beads were added and the lysate was agitated for 1 h at 4 °C. The lysate was centrifuged at 200 × g for 1 min, and the pellet was washed with 400 μl of ice-cold RIPA buffer several times. The pellet was suspended in 60 μl of Laemmli buffer containing β-mercaptoethanol and incubated for 30 min at room temperature. The samples were boiled for 5 min and loaded onto 7.5% SDS-PAGE mini gels. The gel was fixed with 10% acetic acid and 50% methanol for 30 min and dried with a vacuum gel drier at 70 °C for 3 h. The radioiodelabeled proteins were detected and quantified using a PhosphorImager.

**Phosphoamino Acid Analysis—**The conditions for acid hydrolysis and thin layer separation are taken from Boyle et al. (17). In vitro phosphorylation of mOAT was done as described above. The [32P]-labeled protein was resolved by SDS-PAGE and transferred to PVDF. The radiolabeled proteins were detected and quantified using a PhosphorImager.
with orthophosphate in phosphate-free DMEM for 3 h at 37 °C and incubated with the indicated concentrations of okadaic acid for 3 h. Transfected LLC-PK1 cells were labeled with [32P]orthophosphate by autoradiography with a PhosphorImager. The standards were visualized with ninhydrin (Pierce) and the two-dimensional high voltage electrophoresis, for 35 min at 1.2 kV in a 20-cm cellulose thin layer plate. Phosphoamino acids were resolved by mOAT labeling.

RESULTS

Generation of LLC-PK1 Cells Expressing mOAT and mOAT-myc—To make stable mOAT-expressing clones in LLC-PK1 cells, we cloned the cDNAs, encoding mOAT and mOAT tagged with a carboxyl-terminal c-Myc epitope, behind the cytomegalovirus promoter in the mammalian expression vector pcDNA3.1(−), which contains the neo gene for selection with G418. After 2 weeks of selection, 5 clones were obtained from cell populations transfected with the control plasmid (vector alone), 17 clones were obtained from populations transfected pcDNA3.1(−)-mOAT plasmid, and 23 clones were obtained from populations transfected with pcDNA3.1(−)-mOAT-myc plasmid. Preliminary studies measured [14C]PAH uptake in clones of mOAT-expressing and mOAT-myc-expressing cells (data not shown). Clones exhibiting high levels of uptake were chosen for further studies. Northern blot analysis (Fig. 1a) using full-length mOAT cDNA as probe confirmed that clones exhibiting high levels of PAH uptake contained high level of mOAT transcript (lanes 2 and 3), whereas mOAT transcript was not detected in LLC-PK1 cells transfected with vector alone (lane 1). To confirm that these clones expressed high levels of mOAT protein, Western blot studies were performed using crude membrane preparations derived from vector-transfected (control) or mOAT-myc-transfected cells. As shown in Fig. 1b, membrane proteins derived from vector-transfected cells exhibited no reactivity with the c-myc antibody (lane 1), as expected, but membrane proteins derived from cells transfected with mOAT-myc cDNA expressed a c-myc antibody-reactive protein at about 60 kDa (lane 2), consistent with the predicted molecular mass for mOAT (1).

mOAT Localization in Polarized LLC-PK1 Cells—We next examined the localization of mOAT transporter in polarized LLC-PK1 cells. At confluence, LLC-PK1 cells form an epithelium with separate apical and basolateral membrane domains containing different complements of membrane proteins (30). The mOAT transporter in vivo is expressed exclusively at the basolateral membrane (31, 32). The uptake of PAH from either the basal or the apical side was measured in confluent monolayers of cells transfected with vector alone, mOAT, mOAT-myc. Cells were grown on permeable membrane filter supports to provide independent access to either the apical or the basolateral membrane. As shown in Fig. 2a, 20-min uptake of 14C-labeled PAH from the basal side was about 5-fold higher than that from apical side into cells transfected with mOAT or mOAT-myc, whereas little uptake was observed in vector-alone-transfected cells. Therefore, mOAT and mOAT-myc were functionally expressed primarily on the basolateral membrane of polarized LLC-PK1 cells. The time course for 14C-labeled PAH uptake at the basolateral surface was compared in vector-transfected and mOAT-myc-transfected cells (Fig. 2b). As expected, uptake was markedly faster into mOAT-myc-expressing cells. In mOAT-myc-expressing cells, uptake increased linearly for...
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**Fig. 8. Effect of PMA on PAH transport.**

a, confluent monolayers were incubated for 3 h with or without 0.1 μM PMA added directly to the culture media. After washing the cells, 1-min basolateral uptake of [14C]PAH (20 μM) was measured. b, time-course effect of PMA on [14C]PAH uptake. Confluent monolayers were incubated for various periods of time in the absence (open circle) or presence (solid circles) of 0.1 μM PMA. After washing the cells, 1-min basolateral uptake of [14C]PAH (20 μM) was measured. Each point represents the mean ± S.E. of three experiments. c, dose dependence of PMA on [14C]PAH uptake. Confluent monolayers were incubated for 3 h with various concentrations of PMA (10 nM to 1 μM), and 1-min basolateral uptake of [14C]PAH (20 μM) was measured. Values are mean ± S.E. (n = 3).

approximately 2 min and reached a steady state between 5 and 10 min. Therefore, an uptake period of 1 min (initial rate) was chosen for future studies.

To confirm the functional polarization of mOAT at the protein level, we examined the cellular distribution of mOAT protein by confocal microscopy, a procedure that is independent of transporter activity. Previous studies from our laboratory showed that the mOAT-myc protein retained the functional properties of the native (unmodified) structure (2). Our current study (Figs. 1 and 2) confirmed these results. Therefore, the mOAT-myc-transfected cells were chosen for these studies. mOAT-myc-transfected and vector-transfected LLC-PK1 cells were grown to confluence, and mOAT protein was visualized by indirect immunolocalization using confocal laser scanning microscopy (Fig. 3). A top view of the epithelial cell layer showed a clear staining of the plasma membrane of the LLC-PK1-mOAT-myc cells with anti-myc antibody (Fig. 3a), whereas the vector-transfected LLC-PK1 cells showed no detectable labeling (Fig. 3b). Examination of the mOAT-myc-transfected cells at the plane parallel to the permeable filter revealed that the strongest immunostaining was confined to the lateral membrane (Fig. 3d) as compared with the basal membrane (Fig. 3c), whereas the apical membrane exhibited only a weak immunoreactivity (Fig. 3c). Consistent with this result, the z-z analysis (Fig. 3f) showed that, although the apical side was weakly stained, the majority of the immunostaining signal was localized to the basolateral sides. Since the basolateral membrane is the in vivo site of mOAT function, we have focused on the uptake studies at this surface.

**Immunoprecipitation of Phosphorylated mOAT-myc Protein—** SDS-PAGE and autoradiography of immunoprecipitates after metabolic labeling with [32P]orthophosphate of LLC-PK1 cells expressing mOAT-myc revealed that incubation with 1 μM okadaic acid for 3 h significantly increased the intensity of a band centered at ~60 kDa (lane 2), the size expected from the estimated molecular mass for mOAT. The 60-kDa species is absent from immunoprecipitates of cells transfected with pcDNA vector (lane 1) and had received the same treatment in parallel. Together, these findings support the conclusion that mOAT protein is a target for direct phosphorylation by endogenous protein kinases and phosphatases in stably transfected LLC-PK1 cells.

**Effect of Okadaic Acid on mOAT-myc Phosphorylation and Basolateral PAH Uptake—** We then determined the amount of [32P]phosphate incorporation into mOAT as a function of time. As shown in Fig. 5 (a and b), treatment of LLC-PK1 cells with 1 μM okadaic acid induced a time-dependent augmentation of mOAT phosphorylation. To determine the functional consequences of phosphorylation, basolateral PAH transport rate was measured in parallel. The elevation in mOAT phosphorylation induced by okadaic acid displays a similar time course to the okadaic acid-induced down-regulation of [14C]PAH transport (Fig. 5c).

We then determined the amount of [32P]phosphate incorporation into mOAT as a function of dose. As shown in Fig. 6 (a and b), the phosphorylation level of mOAT was elevated with the increase in okadaic acid concentration. Furthermore, the dose response of phosphorylation of mOAT correlated closely with the dose response of okadaic acid-induced down-regulation of [14C]PAH transport (Fig. 6c).

To determine the nature of the phosphorylated residues, we performed phosphoamino acid analysis of mOAT metabolically labeled with [32P] in LLC-PK1 cells and immunoprecipitated with anti-myc antibody. Our results showed that phosphorylation of mOAT occurred on one (or more) serine residues, with little phosphothreonine and no phosphotyrosine detected (Fig. 7).
Effect of PKC Activation on Basolateral PAH Transport and mOAT-myc Phosphorylation—Previous studies have implicated that organic anion transport may be regulated by PKC (9, 12–15). To test whether PKC modulates mOAT function, we treated the mOAT-myc-expressing LLC-PK1 cells with the PKC activator, PMA. Our results showed that, when cell monolayers were treated with 0.1 μM PMA for 3 h, the mOAT-mediated PAH transport was decreased by 50% of that of untreated monolayers (Fig. 8a). This PMA-induced inhibition was time- and concentration-dependent (Fig. 8, b and c).

To clarify the involvement of PKC in the regulation of PAH transport, the effect of various PKC activators on the basolateral PAH transport was studied (Fig. 9). Like PMA, PDBu (the active phorbol ester) and OAG and indolactam (the non-phorbol ester PKC activators) inhibited the PAH uptake. In contrast, 4α-PDD (the inactive phorbol ester that has no effect on PKC) did not affect PAH uptake (Fig. 9a). Staurosporine, a potent inhibitor of PKC, blocked the inhibitory effect by PMA (Fig. 9b).

We then ask whether PKC-dependent inhibition of PAH transport by PMA also occurs, like okadaic acid, via phosphorylation of mOAT. To answer this question, LLC-PK1 cells were metabolically labeled with [32P]orthophosphate using the same experimental conditions that we used for okadaic acid-induced phosphorylation. As shown in Fig. 10 (a and b), no phosphorylated mOAT was detected in the presence of various PKC activators. These results suggest that it is unlikely that phosphorylation of mOAT is responsible for PKC-dependent inhibition of PAH transport.

To further examine the mechanism of the PMA-induced down-regulation of PAH transport, we determined [14C]PAH uptake at different substrate concentrations. An Eadie-Hofstee analysis of the derived data (Fig. 11) showed that pretreatment with PMA resulted in a decreased Vmax (2.0 ± 0.1 pmol/μg-min) with untreated cells, and 1.1 ± 0.1 pmol/μg-min in the presence of PMA) with no significant change in the affinity for PAH (162 ± 25 μM with untreated cells, and 123 ± 19 μM in the presence of PMA). Determination of the protein concentrations in control wells confirmed that PMA treatment did not change the total protein content of the cultures (data not shown).

**DISCUSSION**

To assess the potential involvement of reversible phosphorylation for regulation of PAH transport mediated by mOAT, we have studied the effects of okadaic acid and PKC activators in this process using a heterologous expression system, the pig proximal tubule-like cell line, LLC-PK1. LLC-PK1 cells offer several useful advantages for study of this cloned organic anion transporter. 1) They have many characteristics of proximal renal tubules and have been very useful in understanding other renal epithelial transport processes and cellular functions, including organic cation transport (18). 2) This cell line does not express endogenous PAH transporter (19). Therefore, expression of mOAT in LLC-PK1 cells will allow us to dissect the transport characteristics of mOAT in a relevant mamma-
sensitive dephosphorylation of mOAT, whereas PKC activators inhibited PAH transport by decreasing the maximum transport velocity without direct phosphorylation of the transporter protein. Therefore, the activity of renal organic anion transport is partially determined by the balance between the activities of competing serine-threonine kinases, including PKC and at least one other serine-threonine kinase, and serine-threonine phosphatases, including PP1 and PP2A.

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