Activation of Protein Kinase Cζ Increases OAT1 (SLC22A6) and OAT3 (SLC22A8)-mediated Transport*

Received for publication, October 21, 2008, and in revised form, November 21, 2008. Published, JBC Papers in Press, November 21, 2008, DOI 10.1074/jbc.M808078200

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Organic anion transporters (OATs) play a pivotal role in the clearance of small organic anions by the kidney, yet little is known about how their activity is regulated. A yeast two-hybrid assay was used to identify putative OAT3-associated proteins in the kidney. Atypical protein kinase Cζ (PKCζ) was shown to bind to OAT3. Binding was confirmed in immunoprecipitation assays. The OAT3/PKCζ interaction was investigated in rodent renal cortical slices from fasted animals. Insulin, an upstream activator of PKCζ, increased both OAT3-mediated uptake of estrone sulfate (ES) and PKCζ activity. Both effects were abolished by a PKCζ-specific pseudosubstrate inhibitor. Increased ES transport was not observed in renal slices from OAT3-null mice. Transport of the shared OAT1/OAT3 substrate, ρ-aminophenurate, behaved similarly, except that stimulation was reduced, not abolished, in the OAT3-null mice. This suggested that OAT1 activity was also modified by PKCζ, subsequently confirmed using an OAT1-specific substrate, adefovir. Inhibition of PKCζ also blocked the increase in ES uptake seen in response to epidermal growth factor and to activation of protein kinase A. Thus, PKCζ acted downstream of the epidermal growth factor to protein kinase A signaling pathway. Activation of transport was accompanied by an increase in V_{max} and was blocked by microtubule disruption, indicating that activation may result from trafficking of OAT3 into the plasma membrane. These data demonstrate that PKCζ activation up-regulates OAT1 and OAT3 function, and that protein-protein interactions play a central role controlling these two important renal drug transporters.

Organic anion transporters (OATs) are members of the solute carrier 22A family and play a pivotal role in the renal clearance of small (<500 Dalton) anionic drugs, xenobiotics, and their metabolites. OAT substrates include a variety of drugs such as β-lactam antibiotics, non-steroidal anti-inflammatory drugs, diuretics, and chemotherapeutics (1). OATs are predominantly expressed in renal proximal tubule, with OATs 1–3 localized to the basolateral membrane and OAT4 and URAT1 on the apical membrane. OATs 1 and 3 are dicarboxylate exchangers, and are indirectly coupled to the sodium gradient maintained by Na,K-ATPase through sodium/dicarboxylate co-transport to drive the uphill basolateral step in renal organic anion secretion (2).

Although the ionic gradients, electrophysiology, and underlying kinetics that drive transport by OATs 1 and 3 are well characterized, physiologically important interactions of these basolateral OATs with membrane or cytosolic proteins have yet to be identified (1). Nevertheless, there is clear evidence that other plasma membrane transporters do interact with protein partners, influencing a diverse array of functions including transport itself, cytoskeletal structure, vesicle formation, and trafficking, as well as signaling (3). Among the transporters with activity modulated by protein–protein interactions, particularly by the PDZ proteins, PDZK1 and NHERFs 1 and 2, are apical drug transporters of the SLC22A family, including OCTN1, OCTN2, OAT4, and URAT1 (4–6).

In the present study, we have used a yeast two-hybrid assay to identify putative protein partners that interact directly with OAT3. The C-terminal 81 amino acids of OAT3 were used as bait to screen a human cDNA kidney library. Among the 23 positive clones (putative binding partners) was a clone encoding the C-terminal 141 amino acids of atypical protein kinase Cζ (PKCζ). Functional consequences of the putative OAT3/PKCζ interaction were investigated in rodent renal slices. The resulting data indicate that activation of PKCζ by insulin or epidermal growth factor (EGF) increased OAT3- and OAT1-mediated transport. Thus, PKCζ controls function of both OAT1 and OAT3.

* This work was supported, in whole or in part, by the National Institutes of Health Intramural Research Program from the NIEHS. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡‡1,2 The abbreviations used are: OATs, organic anion transporters; URAT1, urate transporter 1; OATP, organic anion transporting polypeptide; OCTN1, novel organic cation transporter 1; OCTN2, novel organic cation transporter 2; PKCζ, protein kinase Cζ; PKCζ-P5, protein kinase Cζ pseudosubstrate inhibitor; EGF, epidermal growth factor; PKA, protein kinase A; NHERFs, Na+/H+ exchanger regulatory factors; Bt2cAMP, N-6,2’-O-dibutylryladenosine 3’,5’-cyclic monophosphate; ES, estrone sulfate; PAH, para-aminohippuric acid; ADF, adefovir; SNARE, soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor; IP, immunoprecipitate.
major secretory organic anion transporters expressed at the basolateral face of the renal proximal tubule, positioning it to regulate the efficacy of renal drug elimination.

**EXPERIMENTAL PROCEDURES**

**Materials**—Rabbit anti-PKCζ and monoclonal anti-hemagglutinin and anti-tubulin antibodies were obtained from Abcam (Cambridge, MA). Monoclonal anti-myc antibody was obtained from Clontech (Palo Alto, CA). Human recombinant insulin, glutarate, and EGF were obtained from Sigma. PKCζ-pseudosubstrate (PKCζ-PS) inhibitor was purchased from Tocris (Ellisville, MO). PKCζ and PKCθ pseudosubstrate inhibitors and dibutyryl-cyclic AMP (Bt2cAMP) were purchased from EMD Biosciences (San Diego, CA). Tritiated estrone sulfate ([3H]ES; specific activity 50 Ci/mmol) and para-aminohippuric acid ([3H]PAH; specific activity 4.18 Ci/mmol) were obtained from PerkinElmer Life Sciences (Boston, MA) and radiolabeled adeovir ([3H]ADF; specific activity 25 Ci/mmol) was obtained from Moravek Biochemicals (Brea, CA). All other chemicals were purchased from commercial sources at the highest purity available.

**Yeast Two-hybrid Library Screening**—The Matchmaker two-hybrid system from Clontech (Palo Alto, CA) was used according to the manufacturer’s instructions. The C terminus of hOAT3 (243 bp, GenBank™ accession number NM_004254) was cloned into the pGBK7T bait vector (myc-tagged), transformed into AH109 yeast (Alkali Cation Yeast Kit, Qbiogene, Carlsbad, CA), and the bait protein was confirmed by Western blot. The Bait/AH109 yeast were mated with Y187 yeast (QDO, -Ade/-His/-Leu/-Trp) for most stringent selection and the mixture was plated on quadruple dropout YPDA medium transformed with a human kidney cDNA library. The Bait/AH109-Y187 diploid yeast was confirmed by Western blot. The Bait/AH109-Y187 diploid yeast was preincubated at room temperature for 30 min with 15–30 μg/ml insulin, 2 μM PKCζ-PS inhibitor, 75 μM Bt2cAMP, 50 ng/ml EGF, alone or in combination (see figure legends for details). Following preincubation, 30 min uptake of 100 nM [3H]ES, 100 nM [3H]ADF, or 10 μM [3H]PAH was measured at room temperature in the presence of 20 μM glutarate. Slices were rinsed, blotted on Whatman No. 1 paper, weighed, and dissolved in 1 ml of 1 N NaOH overnight on an orbital shaker. Samples were then neutralized with 1 ml of 1 N HCl and assayed for [3H] by liquid scintillation spectroscopy. Transport was calculated as tissue to medium ratios (dpm/mg of tissue ÷ dpm/μl of medium) (2).

**PKCζ Activity**—Kidney slices were homogenized in a buffer (300 mM mannitol, 12 mM Tris/HEPES, pH 7.5) containing a complete protease inhibitor panel from Roche Applied Science. The homogenate was centrifuged at 250 × g for 5 min, yielding a supernatant that was 2–4-fold enriched in plasma membranes. Further enrichment (4–6-fold) was achieved by pelleting the plasma membranes at 20,000 × g for 20 min. Pellets were resuspended in assay buffer containing 0.05% bovine serum albumin, 25 mM MgCl2, 250 mM Tris/HEPES, pH 7.2, with 5 mM dithiothreitol added immediately prior to use. PKCζ activities in kidney membranes were measured using the PKCζ KinEASE™ FP Fluorescein Green Assay (Upstate Signaling Solutions, Lake Placid, NY) and a fluorescence polarization system (Pan Vera, Madison, WI) preprogrammed to measure fluorescein green (excitation 485 and emission at 530 nm). Aliquots of kidney membrane protein, usually 100 μg, were incubated with a phosphorylated tracer in the presence of ATP, magnesium, and a phospho-specific antibody. Reduced binding of the tracer to the antibody reflected competition between tracer and sample PKCζ product. PKCζ activity was calculated from a standard curve for recombinant human PKCζ.

**Preparation of Rat Kidney Basolateral Membrane Vesicles**—Basolateral membrane vesicles were isolated from renal cortex of male Sprague-Dawley CD rats (∼250 g) as previously described (7), using differential centrifugation followed by gradient density centrifugation on an 11% Percoll (GE Healthcare) gradient. The basolateral membrane marker, Na+/K+-ATPase, was enriched 10–20-fold. The brush-border membrane marker, alkaline phosphatase showed enrichments of only 2–3-fold. The final membrane pellet was suspended in vesicle buffer (100 mM mannitol, 100 mM KCl, 20 mM HEPES/Tris, 1 mM MgSO4) at pH 7.4 and stored in liquid nitrogen until use. For kinetic experiments, basolateral membrane vesicles were thawed, centrifuged, resuspended, and allowed to equilibrate in experimental use. Animals were given standard NIH 31 pelleted rodent chow and water ad libitum and maintained at 70°C on a 12-h light/dark cycle. Animals were fasted overnight prior to euthanasia by CO2 narcosis followed by cervical dislocation (mice) or decapitation (rats). The NIEHS Animal Care and Use Committee approved all animal procedures.

**Transport in Rodent Renal Cortical Slices**—Following sacrifice, kidneys were removed and the cortex was sectioned into 0.5-mm slices using a Stadie-Riggs microtome. Tissue slices were maintained in freshly oxygenated ice-cold modified Cross and Taggart saline buffer (95 mm NaCl, 80 mm mannitol, 5 mm KCl, 0.74 mm CaCl2, and 9.5 mm Na2HPO4, pH 7.4). Slices were preincubated at room temperature for 30 min with 15–30 μg/ml insulin, 2 μM PKCζ-PS inhibitor, 75 μM Bt2cAMP, 50 ng/ml EGF, alone or in combination (see figure legends for details). Following preincubation, 30 min uptake of 100 nM [3H]ES, 100 nM [3H]ADF, or 10 μM [3H]PAH was measured at room temperature in the presence of 20 μM glutarate. Slices were rinsed, blotted on Whatman No. 1 paper, weighed, and dissolved in 1 ml of 1 N NaOH overnight on an orbital shaker. Samples were then neutralized with 1 ml of 1 N HCl and assayed for [3H] by liquid scintillation spectroscopy. Transport was calculated as tissue to medium ratios (dpm/mg of tissue ÷ dpm/μl of medium) (2).
TABLE 1
Subset of putative OAT3 binding partners

| Category                              | Identities                                      | Protein domains/ontologies                        |
|---------------------------------------|------------------------------------------------|--------------------------------------------------|
| Vesicle formation, protein trafficking| Pantophysin, synaptophysin-like protein (H-SP1)  | MARVEL, transport vesicle biogenesis              |
|                                       | GTPase Rab14                                    | Ras superfamily, small GTPase, vesicular trafficking, signal transduction |
|                                       | GTPase Rab1B                                    | Ras superfamily, small GTPase, vesicular trafficking, signal transduction |
|                                       | SNARE associated protein snapin (SNAPAP)        | SNARE binding, synaptic vesicle docking and fusion |
| Transport and metabolism              | Dna1 (Hsp40)                                    | Dna1 superfamily, chaperone associated with Hsp70 system |
|                                       | Aspartoacylase 2, aminoacylase 2 (ASPA)          | Succinylglutamate desuccinylase/aspartoacylase, amino acid transport and metabolism |
| Signaling                             | AUP1 homolog                                    | Phospholipid/glycerol acyltransferase, CUE, lipid transport and metabolism |
|                                       | PKCζ                                            | Serine/threonine protein kinase, phosphotransferase |

TABLE 2

goat anti-rabbit IgG (Pierce) for 1 h. SuperSignal West Pico Chemiluminescent Substrate (Pierce) was utilized to detect antibodies, and a Gel DocTM XR system (Bio-Rad) was utilized to detect proteins.

Statistics—All experiments were repeated at least three times using preparations from separate animals. Data are expressed as mean ± S.E. Means were compared using the unpaired, two-tailed Student’s t test. In kinetic experiments, differences were analyzed using two-way analysis of variance with randomized-block design. p values ≤0.05 were deemed to be significant.

RESULTS

Yeast Two-hybrid Assay—Using the 81 C-terminal amino acids of OAT3 as bait and a GAL4-based reporter system, a human cDNA kidney library was screened to identify putative binding partners. Following an initial 21-day incubation and two replicate rounds of plating on quadruple dropout YPD medium (-Ade/-His/-Leu/-Trp, most stringent selection conditions), 23 robust diploid colonies were selected for identification. A subset of the putative OAT3 binding partners is shown in Table 1 and includes proteins relevant to vesicle formation, protein trafficking, transport, metabolism, and signaling. Of particular interest was PKCζ because it is a signaling protein affecting many cellular functions (8). The interaction of the OAT3 C terminus with the C-terminal 141 amino acids of PKCζ was further validated in yeast by co-transformation and additional matings using the individual OAT3 bait and isolated PKCζ prey constructs. The presence of OAT3 bait in AH109 haploid and PKCζ prey/AH109-Y187 diploid yeast was confirmed by Western blot (Fig. 1c).

Association of OAT3 and PKCζ—Direct physical association between rPKCζ and rOat3 was demonstrated by immunoprecipitation from rat kidney lysates. As shown in Fig. 2a, precipitation with anti-PKCζ pulled down both rPKCζ itself and rOat3. Similar results were obtained upon initial precipitation with anti-rOat3 antibody (Fig. 2b).

Effect of PKCζ Activity on OAT3-mediated Transport—The putative OAT3/PKCζ interaction was investigated in isolated rodent renal cortical slices, using insulin as an upstream activator of PKCζ (9). To maximize sensitivity to insulin treatment, animals were fasted overnight prior to sacrifice. Uptake of 100 nM [3H]ES, a prototypical organic anion that is preferentially transported by OAT3 (10, 11), was measured in rat and mouse renal cortical slices. As shown in Fig. 3a, treatment with human recombinant insulin (30 μg/ml for rat; 15 μg/ml for mouse) significantly increased uptake of [3H]ES in slices from both species. This increase was prevented by exposure to 2 μM PKCζ-PS.
inhibitor during preincubation, suggesting that specific activation of PKCζ was responsible for the increased ES uptake, presumably mediated by OAT3 (see below). Activation of PKCζ requires the phosphorylation of two key threonine residues, Thr410 and Thr560 (9). An assay based on this observation showed that the activity of PKCζ paralleled the observed changes in transport activity, increasing markedly upon insulin treatment and returning to control levels in the presence of the PKCζ-PS inhibitor (Fig. 3b). The insulin effect on ES uptake was not altered by pseudosubstrate inhibitors for other PKC isozymes (η and θ, of the “novel” subclass, requiring diacylglycerol but not Ca2+ for activation) (Fig. 3c).

**OAT Transporters Affected by PKCζ**—Although estrone sulfate is primarily transported by OAT3 in the kidney (10), a number of other basolateral transporters could contribute to its uptake in the rodent kidney (1, 12, 13). To confirm that the increase in ES transport was mediated by OAT3, transport experiments were repeated using renal cortical slices from the OAT3-null mouse, previously described by Sweet et al. (10). In contrast to the increase in ES uptake observed in wild-type mice and rats, insulin did not stimulate ES uptake in renal slices from OAT3-null mice (Fig. 4a), demonstrating that OAT3 was responsible for the observed stimulation of ES transport in the wild-type animals. However, uptake of PAH, a substrate for...
both OAT1 and OAT3, was stimulated by insulin in renal slices from wild-type and slightly in OAT3-null mice (Fig. 4b). These results suggest that OAT1 might also be regulated by PKCζ. To address this possibility, transport of [3H]ADF was evaluated in rat renal slices. Adefovir, an antiviral, acyclic nucleoside phosphonate, has previously been shown to be effectively transported by OAT1, but not by OAT3 (11, 14, 15). As shown in Fig. 4c, transport of [3H]ADF by rat renal slices was once again stimulated by insulin, and this effect was blocked by the PKCζ-PS inhibitor. Interestingly, mOat1 does not transport adefovir, unlike hOAT1 and rOat1 (11). Thus, in mouse renal slices, adefovir uptake was not stimulated (Fig. 4c), indicating that insulin stimulation of adefovir uptake in rat kidney depends specifically on the ability of rOat1 to transport this substrate.

Effect of EGF on OAT3 Transport—Soodvilai et al. (16) found that treatment of isolated rabbit renal proximal tubules with EGF increased OAT3-mediated uptake of estrone sulfate. EGF was shown to stimulate OAT3 transport via a mitogen-activated protein kinase to phospholipase A2 to prostaglandin E2 to protein kinase A (PKA) pathway, although the mechanism of stimulation was not determined. As shown in Fig. 5, preincubation of mouse renal slices with EGF (50 ng/ml) significantly increased ES uptake in wild-type mice. No stimulation was observed in OAT3-null animals. In wild-type animals, EGF stimulation was blocked by the PKCζ-PS inhibitor, indicating that PKCζ also contributes to OAT3 activation via the EGF to PKA signaling pathway.

To assess where PKCζ acted in this pathway, we confirmed that dibutryl-cAMP-mediated activation of PKA led to the expected increase in ES transport by rat slices and that stimulation was prevented by the PKA inhibitor, H89 (16, 17). In our slice system, 30 min preincubation with 75 μM Bt2cAMP doubled ES uptake and this effect was abolished by 100 μM H89 (data not shown). Moreover, as shown in Fig. 6a, both H89 and PKCζ-PS blocked EGF stimulation and simultaneous exposure to both inhibitors yielded no increase in inhibition over that seen with either inhibitor alone. Finally, as shown in Fig. 6b, these inhibitors produced an identical inhibitory profile when used to block insulin-stimulated ES uptake. Together these data indicate that PKCζ is situated between PKA and OAT1/OAT3 in activation of transport via either insulin or EGF signaling.

**Apparent Role of Trafficking in Transport Activation**—As shown in Table 2, insulin activation of OAT3-mediated ES transport was characterized by a 2–3-fold increase in V_max for both the OAT3 substrate, ES, and the OAT1 substrate, adefovir. This suggested that additional transporters may have been trafficked to the membrane. Therefore, nocodazole, brefeldin A, and cytochalasin D were used to disrupt microtubules/microfilaments and reduce trafficking. All three inhibitors abolished insulin-induced stimulation of ES transport (Fig. 7). These agents had no effect on baseline transport in the absence of insulin.

**DISCUSSION**

Characterization of membrane transporters has traditionally focused on functional and physiological studies in vivo or in
of protein-protein interactions have also been observed for apically expressed members of the OAT family. Most notably, interactions of URAT1 with PDZK1 altered the efficacy of urate transport across the apical membrane of renal cells in culture (4, 20). Finally, signaling through activation of conventional PKC isoforms has been shown to decrease the activity of human, rat, and rabbit OATs 1 and 3 in several in vitro expression systems (16, 21–25).

To assess potential protein-protein interactions for the basolateral OATs that mediate the initial, energy-dependent step in secretion of drugs and toxins, we used a yeast two-hybrid assay to identify putative OAT3-associated proteins in the kidney. We found interactions between the 81 C-terminal amino acids of OAT3 and 23 peptide fragments from a human cDNA kidney library (Table 1). A number of these putative hOAT3-binding proteins appeared relevant to transport and metabolism (heat shock protein; aspartoacylase 2) or to vesicle formation and trafficking (H-SP1, pantophysin; RAB14, small GTPase; SNAPAP, SNARE-associated protein). Others were associated with signal transduction (RAB14, RAB1B, and PKCζ). As discussed below, we have focused on PKCζ in the current experiments and detailed assessment of the importance of other candidate proteins in hOAT3 function and/or subcellular localization must await additional study.

Unlike both conventional and novel PKCs, PKCζ is an atypical isoform, i.e. it is insensitive to Ca\(^{2+}\) and unresponsive to diacylglycerol and phorbol esters. Instead, PKCζ is activated by phosphatidylinositol trisphosphate, phosphatidylyserine, arachidonic acid, ceramide, and insulin (8, 9, 26). Activity of PKCζ is inhibited by pseudo-substrate peptides, staurosporine, wortmannin, and LY294009 (8, 9). Importantly, PKCζ is known to be involved in a variety of signaling pathways including mitogen-stimulated cell growth (mitogen-activated protein kinase

heterologous in vitro expression models. More recently, the emergence of new molecular technologies has prompted investigations into the molecular biology and interactions of transporters with regulatory molecules, notably via distinct protein-protein interactions that modulate transporter structure, activity, trafficking, or signaling. Biber and Murer and co-workers (18, 19) have shown that interactions with PDZ domain proteins were important for both apical expression and regulation of renal phosphate transporters. Similar regulatory effects
Modulation of OAT-mediated Transport by PKCζ

cascade, p70 S6 kinase; in cell growth and survival (NFκB activation); and in generation of cell polarity (8). We chose to investigate the putative OAT3/PKCζ interaction in a fresh renal tissue preparation, the renal cortical slice, rather than in a culture system, ensuring the presence of all normal regulatory pathways. Our working hypothesis was that manipulation of PKCζ activity would modulate normal OAT3-mediated transport. As shown in Fig. 3a, rat and mouse renal slices preincubated with insulin showed a significant increase in uptake of estrone sulfate, and co-administration of a PKCζ-specific pseudo-substrate inhibitor completely blocked the effect of insulin, strongly supporting a specific role for PKCζ. The induction and inhibition of PKCζ activity was confirmed by enzyme activity assays in renal tissue lysates (Fig. 3b). In addition, pseudosubstrate inhibitors of PKCη and PKCθ, both of the novel subclass of PKC isozymes, did not affect the insulin effect on ES uptake, further demonstrating the unique role of PKCζ in this physiological response (Fig. 3c).

When uptake experiments were repeated in renal slices from OAT3-null mice, no effects on ES transport were observed (Fig. 4a), clearly documenting the involvement of the OAT3 isoform. Additional experiments with the OAT1-specific substrate, adefovir (11, 14, 15), indicated that this basolateral transporter was controlled by PKCζ, just like OAT3 (Fig. 4c). Although we have not directly addressed interactions of renal organic anion transporting polypeptides (OATPs of the SLO or SLC21A families) that might transport ES (27, 28), the loss of response to insulin or EGF (Figs. 4 and 5) in the OAT3-null mice and the lack of stimulation of adefovir uptake by mouse slices (because mOat1 does not transport adefovir) argues that any OATP contribution in these studies must be minimal.

As shown in Fig. 5, EGF treatment of renal slices from wild-type mice also increased ES uptake. These results were entirely comparable with the findings of Soodvilai et al. (16) in isolated rabbit renal proximal tubules. Because activation of PKCζ by EGF treatment has been seen in human breast cancer cells (29) and Caco-2 cells (30), it seemed probable that, like the insulin effect discussed above, EGF stimulation was mediated through PKCζ. Indeed, as shown in Fig. 5, EGF-stimulated ES transport by the mouse kidney slices was (a) completely blocked by PKCζ-PS inhibitor and (b) not found in slices from OAT3-null mice. Furthermore, consistent with results in the rabbit (15), we also demonstrated that Oat3 activity could be manipulated by PKA activation/inhibition, and that the upstream stimulation by either EGF or insulin was blocked by both H89 (PKA inhibitor) and PKCζ-PS (Fig. 6); strongly implicating PKCζ as the end-mediator in this signaling pathway.

Overall, the data presented here indicate that PKCζ plays a central role in up-regulation of both OAT1 and OAT3 transport, thus, increasing net secretory transport of drugs and xenobiotics from the body. On the other hand, there is substantial evidence that activation of the conventional PKCs leads to a down-regulation in the activities of these transporters (15, 16, 22–24). For example, activation of conventional PKCs by phorbol ester treatment led to reduced transport in Xenopus oocytes expressing hOAT1 (24). This effect appeared to be mediated by trafficking of the transporter out of the plasma membrane, and did not depend on phosphorylation of the transporter. Phenytoine also produced a comparable decrease in transport in the intact tubules of the rabbit (15), demonstrating that down-regulation is not limited to transport in expression systems. Our data also indicates that increased OAT transport may be mediated by changes in trafficking to the plasma membrane (Table 2 and Fig. 7), paralleling the findings of Wolfe et al. (24) for down-regulation of OAT activity following activation of conventional PKCs. Together, these two lines of evidence outline an overall regulatory system in which activation of the conventional PKC isozymes leads to reduced basolateral OAT transport and activation of PKCζ leads to their up-regulation.

In summary, an interaction between PKCζ and OAT3 was identified through yeast two-hybrid analysis. PKCζ activity and OAT3-mediated transport of ES were shown to be increased following insulin or EGF treatment and both were completely inhibited by PKCζ-PS. Together these data provide clear evidence that protein–protein interaction via this protein kinase may up-regulate organic anion transport. Thus, it appears that the insulin (or EGF) to PKCζ signaling pathway described here is one arm of an overall regulatory system in which activation of PKCζ leads to up-regulation of OAT1 and OAT3 transport; whereas, activation of the conventional PKC isoforms leads to their down-regulation (16, 23, 24). Indeed, given the complexity of membrane transporter architecture and function, it is likely that PKCζ is but one of many regulatory proteins capable of altering OAT function, suggesting that characterization of additional OAT protein–protein interactions will prove important for better understanding of both transporter function and drug pharmacology in the kidney.

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