Involvement of tyrosine kinase and PI3K in the regulation of OAT3-mediated estrone sulfate transport in isolated rabbit renal proximal tubules

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The kidney plays an important role in the excretion of a structurally diverse array of compounds from the body. A great number of the potentially toxic compounds in the human body are organic anions, including both endogenous compounds and xenobiotics that entail a variety of environmental toxins and pharmaceutical agents (5, 13, 30, 47, 48, 51). The active secretion of anionic compounds to the tubular lumen appears to be restricted to the renal proximal tubule. This process involves the following steps: transport of compounds from the blood into the cells across the basolateral membrane against an electrical gradient established by Na-K-ATPase (28, 29, 49). The electrochemical gradient and movement from the cells into the lumen down an electrochemical gradient (30, 51). Transport into the cell at the basolateral membrane, which is the rate-limiting step in secretion, is a tertiary active process. This step involves countertransport of an organic anion into the cell against its electrochemical gradient in exchange for mediated efflux of α-ketoglutarate (α-KG). The outwardly directed gradient for α-KG is not only maintained by intracellular metabolic generation of α-KG but is also fueled by active αKG uptake across the basolateral membrane via a Na-dicarboxylate cotransporter (NaDC) that is, in turn, driven by the transmembrane sodium gradient established by Na-K-ATPase (28, 29, 49).

Many cDNAs encoding renal organic anion transporters (OATs) have been cloned, including OAT1, OAT2, OAT3, OAT4, OAT1P1, OAT-K1, and OAT-K2 (51). The basolateral transport of organic anion across the proximal tubular cells appears to involve two distinct processes including OAT1 and OAT3 (23, 26, 43). OAT3 shows high amino acid sequence identity with OAT1 (about 49%) (8, 24). Moreover, OAT1 (36, 44) and OAT3 (2, 42) share a common energetic mechanism (i.e., OA/α-KG exchange) and have overlapping substrate specificities (5, 45). However, activity of the two transporters in rabbit renal proximal tubules can be distinguished using “homologue-selective substrates”: estrone sulfate (ES) transport is effectively restricted to OAT3, whereas p-aminophenylarbutin (PAH) transport is effectively restricted to OAT1 (25).

Basolateral organic anion transport in renal cells has been shown to be regulated by several hormones that activate protein kinases, including bradykinin, phenylephrine, and parathyroid hormone (19, 27, 39). The phosphorylation cascades induced by protein kinases result in either the activation or inhibition of specific enzymes or the activation of the transcription of specific genes (47). Activation of protein kinase C (PKC) by phorbol 12-myristate 13-acetate (PMA) and 1, 2-dioctanoyl-sn-glycerol (DOG) downregulates organic anion transport mediated by rat OAT3 (rOAT3) (46). In addition, activation of PKC, either directly with PMA or indirectly with ligands of physiological receptors coupled to the PKC pathway (e.g., the α1-receptor agonist, phenylephrine), inhibits basolateral uptake of ES in the S2 segment of rabbit renal proximal tubules (41).

Recent data indicate that epidermal growth factor (EGF), which appears to be important in normal tubulogenesis and tubular regeneration after injury, stimulates basolateral uptake of PAH in cell cultures (33, 34). In addition, EGF stimulates PAH and ES transport in intact renal proximal tubules (32, 41). This effect of EGF on the basolateral uptake of organic anions occurs via the mitogen-activated protein kinase (MAPK) pathway. Administration of EGF leads to sequential activation of mitogen-activated/extracellular signal-regulated kinase kinase (MEK), extracellular signal-regulated kinase 1 and 2 (ERK1/2), and phospholipase A2 (PLA2), resulting in an increased release of arachidonic acid. Arachidonic acid is subsequently metabolized to prostaglandin via cyclooxygenase 1 (COX1), which then mediates EGF-induced stimulation of basolateral PAH uptake (34). In S2 segments of rabbit renal proximal tubule, prostaglandin E2 (PGE2) enhances basolateral PAH and ES uptake via adenylyl cyclase activation and causes protein kinase A (PKA) activation (32, 41).
At present, although substantial information concerning the regulation of organic anion transport mediated by OAT3 in heterologous systems is available; little is known about the regulation of organic anion transport in the intact renal proximal tubule. It is imperative to study regulation of organic anion transport with the transporter expressed in its native, intact system to be certain that regulatory responses observed in other systems reflect, in at least a qualitatively significant way, those occurring under comparatively normal physiological circumstances.

Tyrosine kinase and phosphatidylinositol 3-kinase (PI3K) have been shown to be involved in the signal transduction of virtually all growth factors and cytokines including EGF, insulin, insulin-like growth factor (IGF), and platelet-derived growth factor (PDGF), in kidney cell lines (3, 4, 11, 12, 16, 37). They play an important role in the kidney; for instance, PI3K and tyrosine kinase stimulate PAH transport in proximal tubules (17). In addition, PI3K and tyrosine kinase activate sodium bicarbonate cotransport in OK cells and sodium-hydrogen exchange (NHE3) in MKCC and OKP cells (3, 12, 14, 20, 31). In a previous study, we demonstrated that OAT3 activity might be regulated differentially by protein kinases (41). In the present study, therefore, we investigated whether tyrosine kinase and PI3K, which have been shown to regulate several cellular functions in renal proximal tubules, modulate organic anion transport via OAT3 in nonperfused S2 segments of rabbit renal proximal tubules. Inhibitors of tyrosine kinase (genistein) and PI3K (wortmannin) and a physiological stimulus (EGF) of tyrosine kinase and PI3K were used to modulate OAT3 activity. We present data indicating that inhibition of tyrosine kinase and PI3K downregulates OAT3 activity.

METHODS

Chemicals. [3H]ES (43.5 Ci/mmol) was purchased from Perkin Elmer Life Science Products (Boston, MA). EGF, genistein, and wortmannin were purchased from Sigma (St. Louis, MO), and U-0126 was purchased from Promega (Madison, WI). Antibody against the phosphorylated form of ERK1/2 was purchased from Cell Signaling Technology (Beverly, MA). All other chemicals were purchased from standard sources and were generally of the highest purity available.

Preparation of isolated tubules. New Zealand white rabbits [1.5–2.0 kg, National Laboratory Animal Center (NLAC), Bangkok, Thailand] were killed by intravenous injection of pentobarbital sodium, and all protocols employing rabbits were conducted in accordance with principles and guidelines of the Laboratory Animal Ethical Committee of Mahidol University, Bangkok, Thailand. The kidneys were flushed via the renal artery with an ice-chilled HEPES-sucrose buffer containing 250 mM sucrose and 10 mM HEPES, adjusted to pH 7.4 with Tris (hydroxymethyl)aminomethane hydrochloride, and bubbled with 100% O2 before use. They were then gently removed and sliced transversely using a single-edge razor. A kidney slice was transferred to the lid of a plastic petri dish on ice that contained the standard solution (nutritionally enriched bicarbonate buffer) used for dissecting and bathing tubules (mM: 110 NaCl, 25 NaHCO3, 5 KCl, 2 NaH2PO4, 1 MgSO4, 1.8 CaCl2, 10 Na-acetate, 8.3 d-glucose, 5 l-alanine, 0.9 glycine, 1.5 lactate, 1 malate, and 1 sodium citrate). This standard solution was aerated continuously with 95% O2-5% CO2 to maintain the pH at 7.4. The osmolality of the solutions averaged ~290 mosmol/kgH2O.

S2 segments of proximal tubules were individually dissected from the cortical zone without the aid of enzymatic agents as described by others (6). All dissections were performed at 4°C, and all experiments were performed at 37°C. Transport rates were normalized to tubule surface area based on tubule lengths and average diameters as determined from photographs taken through a dissecting microscope equipped with a digital image capture system (Snappy, Play).

Preparation of suspensions of RPT. Suspensions of rabbit renal proximal tubules were isolated and purified from New Zealand White rabbit kidneys (1.5–2 kg) as previously described (22). The final tubule pellet was resuspended and incubated with protein kinase activator or inhibitor before Western blot analysis was performed.

Measurement of transport of [3H]ES in S2 segments of nonperfused, isolated renal proximal tubule. These experiments were performed in a manner similar to that used previously (9, 10, 22). Briefly, an appropriate number of tubule segments (3–5 for each condition to be studied) were teased from fresh renal tissue and maintained in oxygenated (95% O2-5% CO2), nutritionally enriched bicarbonate buffer solution at 4°C that was covered with a layer of mineral oil to prevent evaporation before use. The tubules were then transferred to baths containing the different activators or inhibitors of protein kinases at 37°C. At the end of this preincubation period, each tubule was transferred to a new bathing medium containing [3H]ES (~0.12 μM) for an uptake of 30 s. This time period was chosen because in preliminary experiments it was found to permit an adequate approximation of the initial rate of ES uptake. No putative stimulators or inhibitors were present in the medium during these 30-s uptakes. Uptake was stopped by transferring each tubule into individual wells containing 7 μl N NaOH. Substrate accumulated by each tubule was determined by liquid scintillation counting. Control and experimental uptakes in the tubules were determined alternately and sequentially in tubules from the same kidney.

Western blot analysis. The tubule suspension was rinsed three times with nutritionally enriched bicarbonate buffer and then incubated with control medium or media containing protein kinase activators or inhibitors. Subsequently, tubules were washed with ice-cold control medium and lysed in iced cold Triton X-100 lysis buffer (50 mM Tris-HCl, 100 mM NaCl, 50 mM NaF, 5 mM EDTA, 40 mM β-glycerophosphate, 200 μM sodium orthovanadate, 100 μM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μM pepstatin A, 1% Triton X-100) for 25 min at 4°C. Insoluble material was removed by centrifugation at 10,000 rpm for 20 min at 4°C. Equal amounts (80 μg) of protein from the tubule lysate were separated by 10% SDS-PAGE and transferred to a Hybond-ECL nitrocellulose membrane (Amersham Biosciences). The membrane was incubated in 5% nonfat milk for 1 h and then blotted with rabbit anti-phosphorylated ERK1/2 antibody (pERK1/2, p42/44) (Cell Signaling Technology) in a 1:1,000 dilution at 4°C overnight. After incubation with the primary antibody, the membrane was washed three times for 5 min each and incubated for 1 h at room temperature with a 1:5,000 dilution of secondary antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG. After the membrane was washed three times, immunoreactivity was detected by chemiluminescence and was visualized by using enhanced chemiluminescence Western blot reagents and hyperfilm ECL (Amersham Biosciences). Western blotting was performed with protein from five independent extractions from five rabbits. The membranes were stripped by incubating in 62.5 mM Tris-HCl, 2% SDS, and 100 mM β-mercaptoethanol at 70°C for 30 min. The membranes were reprobed with mouse anti-β-actin to normalize the protein content in each lane. For quantification of ERK1/2 phosphorylation, films were scanned and volume integration was performed using Image Scanner model Labscan V3.01 and ImageMaster TotalLab V1.00 (Amersham).

Data analysis. Data are summarized as means ± SE. The n value indicates the number of experiments (each using tubules from a different rabbit). The differences between rates of uptake for the different regimens were analyzed with the one-way ANOVA. In all analyses, differences were considered statistically significant when P < 0.05.
compared with control value. Genistein, an inhibitor of tyrosine kinase (1, 17). Figure 1 shows a dose-response plot of the genistein effect on basolateral uptake of estrone sulfate (ES) in nonperfused S2 segments of rabbit renal proximal tubule. The tubules were preincubated with control medium containing [3H]ES (0.12 nM) for 30 s. Genistein inhibited ES transport in a dose-dependent manner, with 1 μM proving to be the smallest effective concentration (decrease of 21 ± 6%, compared with control). Increasing the concentration of genistein to 50 μM led to further inhibition (decrease of 49 ± 7% of control). To determine whether the inhibitory effect produced by genistein on basolateral uptake of ES reflected inhibition of tyrosine kinase and was not produced from a nonspecific effect of genistein, we examined the effect of daidzein, an inactive analog of genistein, which does not exhibit the inhibitory effect on tyrosine kinase activity. Exposing tubules to 50 μM daidzein for 20 min produced no effect on the initial rate of ES uptake as shown in Fig. 1B. These data indicated that the inhibitory effect produced by genistein reflected an inhibition of tyrosine kinase activity.

Effect of PI3K inhibition by wortmannin on basolateral uptake of ES. PI3K plays a role in cellular functions in proximal tubule and regulates the activity of transporters including the sodium bicarbonate cotransporter, NHE3, and OAT1 (3, 14, 17). Consequently, it is possible that PI3K regulates the transport of organic anions mediated by OAT3. To test this hypothesis, we examined the effect of wortmannin, a specific inhibitor of PI3K, on the initial rate of ES uptake of ES. The tubules were preincubated with control medium containing increasing concentrations of wortmannin for 20 min and then incubated with control medium containing [3H]ES (0.12 nM) for 30-s uptake. Exposing renal proximal tubules to wortmannin at 100 nM significantly reduced the uptake of ES by 18 ± 3% of control (Fig. 2), and increasing the concentration to 1 μM led to a further inhibition (decrease of 28 ± 8% of control).

Effect of genistein and wortmannin on EGF-induced stimulation of ES uptake. Previously, we reported that EGF stimulates ES uptake in intact renal proximal tubules (41). To investigate whether tyrosine kinase and PI3K-mediate the EGF effect on OAT3 activity, we examined the effect of genistein or wortmannin during EGF stimulation. First, we examined the
Effect of genistein during the time when the tubules were exposed to EGF. The tubules were preincubated under four conditions:

1) control medium;
2) control medium containing 50 ng/ml EGF for 10 min;
3) control medium containing 10 μM genistein for 20 min; and
4) control medium containing 10 μM genistein for 10 min followed by control medium containing 50 ng/ml EGF and 10 μM genistein for 10 min. The results are shown in Fig. 3 A. As expected, EGF stimulated ES uptake, whereas genistein inhibited ES uptake. This stimulatory effect produced by EGF was eliminated by exposure to genistein.

Next, we determined the effect of wortmannin on the EGF stimulation. The experimental protocol was the same as that described above. The tubules were preincubated under four conditions:

1) control medium;
2) control medium containing 50 ng/ml EGF for 10 min;
3) control medium containing 100 nM wortmannin for 20 min; and
4) control medium containing 100 nM wortmannin for 10 min followed by control medium containing 50 ng/ml EGF and 10 μM genistein for 10 min. Again, as expected, EGF stimulated ES uptake, whereas wortmannin inhibited ES uptake. The stimulatory effect of EGF on ES uptake was significantly reduced in the presence of 100 nM wortmannin (Fig. 3B). These results support the idea that the stimulatory effect of EGF is mediated by tyrosine kinase and PI3-K.

Fig. 3. Effect of genistein (A) and wortmannin (Wort; B) on the initial rate of ES uptake during stimulatory effect of epidermal growth factor (EGF). The conditions of preincubation are given under each bar (see details in text). The results are expressed as means ± SE percentage of control. The numbers in the bars indicate number of experiments (each experiment using tubules from a different rabbit). Mean control initial rates of ES uptake were 121 ± 7 fmol·min⁻¹·mm⁻² (A) and 134 ± 17 fmol·min⁻¹·mm⁻² (B). *P < 0.05 compared with control. **P < 0.05 compared with EGF-treated group.

Fig. 4. A: effect of U-0126 and genistein on initial rate of ES uptake. B: effect of U-0126 and wortmannin on initial rate of ES uptake. The conditions of preincubations are given under each bar (see detail in text). The results are presented as means ± SE percentage of control for 4 experiments (each using tubules from a different rabbit). Mean control initial rates of ES uptake were 100 ± 7 fmol·min⁻¹·mm⁻² (A) and 148 ± 20 fmol·min⁻¹·mm⁻² (B). *P < 0.05 compared with control value.
37 ± 3 and 29 ± 9% of control value, respectively. When the tubules were exposed to medium containing U-0126 plus genistein, the depression of ES uptake was not significantly different from that occurring when the tubules were exposed to U-0126 or genistein alone (31 ± 8 vs. 37 ± 3, 29 ± 9%). The lack of an additive effect suggests that the action of tyrosine kinase involves the same pathway(s) as MAPK.

Second, we also investigated whether inhibition of both PI3K and MEK produced an additive effect on ES uptake. The tubules were preincubated under four conditions: 1) control medium; 2) control medium containing 10 μM U-0126 for 20 min; 3) control medium containing 1 μM wortmannin for 20 min; and 4) control medium containing 10 μM U-0126 plus 1 μM wortmannin for 20 min. In this study we used concentration of wortmannin at 1 μM to be certain that the additive effect of the inhibitors could be observed if PI3-K regulating of OAT3 activity is not part of the MAPK pathway. It was found that the inhibition produced by U-0126 plus wortmannin was not significantly different from that produced by U-0126 or wortmannin alone (36 ± 10 vs. 34 ± 10, 35 ± 8%) as shown in Fig. 4B. The results indicate that PI3-K also involves the same pathway(s) as MAPK.

Our previous and current observations indicate that EGF acts via ERK, tyrosine kinase, and PI3K in intact renal proximal tubules to stimulate OAT3-mediated organic anion transport. This then raised the question of how they work. To determine whether tyrosine kinase or PI3K was upstream or downstream of ERK activation in the regulation of OAT3 activity, we examined the effect of these compounds on phosphorylation of ERK in a preparation of isolated, suspended rabbit renal proximal tubules [separate studies showed that ES uptake into these tubules responds to EGF in a manner similar to that observed for isolated single rabbit proximal tubules (data not shown)]. The tubule suspensions were preincubated under six conditions: 1) control medium; 2) control medium containing 50 ng/ml EGF for 10 min; 3) control medium containing 10 μM genistein for 20 min; 4) control medium containing 10 μM genistein for 10 min followed by control medium containing 50 ng/ml EGF plus 10 μM genistein for 10 min; 5) control medium containing 1 μM wortmannin for 20 min; and 6) control medium containing 1 μM wortmannin for 10 min followed by control medium containing 50 ng/ml EGF plus 1 μM wortmannin for 10 min. As shown in Fig. 5, EGF stimulated ERK1/2 phosphorylation, whereas wortmannin itself slightly inhibited phosphorylation of ERK1/2, although the effect was not significantly different from the control level. The EGF-induced ERK1/2 phosphorylation was reduced by wortmannin suggesting that the EGF stimulation of PI3K was upstream of the MAPK signaling pathway in the overall process of EGF-stimulated ES uptake in intact renal proximal tubules. In contrast to wortmannin, genistein had no effect on EGF-induced ERK1/2 phosphorylation. These results imply that tyrosine kinase-mediated EGF stimulation of OAT3 activity is downstream of the MAPK signaling pathway.

DISCUSSION

Tyrosine kinase and PI3K play an important role in a number of kidney functions. These protein kinases have been reported to regulate the activity of several transport processes, including the sodium bicarbonate cotransporter, NHE3, and OAT1 (3, 12, 14, 17). In a previous study, we demonstrated that OAT3 is differentially regulated by several protein kinases including PKC, PKA, and MAPK (41). In this study, we investigated the effect of tyrosine kinase and PI3K on the OAT3-mediated organic anion transport in intact renal proximal tubules. Genistein, which is a broad cytoplasmic tyrosine kinase inhibitor, inhibited ES uptake. This was not due to a nonspecific effect of genistein as shown by the fact that exposure to daidzein, an inactive analog of genistein that does not exhibit tyrosine kinase inhibitory activity (38), had no effect on the initial rate of basolateral uptake of ES (Fig. 1B). However, 32 forms of nonreceptor-mediated tyrosine kinase have been found in the renal cell (40), including, FES, TEC, ABL, SRC, JAK1, SYK, FAK, ACK, and TK1. Therefore, it is unclear which specific forms of tyrosine kinase are responsible for the inhibition of OAT3 activity.

Wortmannin, a specific inhibitor of PI3K, also inhibited OAT3 activity in a dose-dependent manner (Fig. 2). These data imply that under physiological conditions, OAT3 activity is under the tonic influence of both tyrosine kinase and PI3K activities. PI3Ks are a class of enzymes that phosphorylate phosphatidylinositol and its derivatives. Phosphatidylinositol plays an important role in signal transduction and is the precursor to many second messenger molecules. PI3K activity increases in response to growth factors and hormones. These include colony-stimulating factor 1 (CSF-1), insulin-like growth factor 1 (IGF-1), platelet-derived growth factor (PDGF), and EGF. PI3Ks are organized into three classes on
the basis of structure, substrate specificity, and mode of regulation (21), and it is unclear which class of PI3K-regulated OAT3 activity is in our study.

Basolateral uptake of ES by OAT3 is a tertiary active process. This process involves the parallel activity of three transport processes including organic anion/α-KG exchange, Na-α-KG cotransport, and the Na-K-ATPase transport of sodium. Modulation of any of these three processes may influence the basolateral uptake of ES-mediated by OAT3. Indeed, modulation of the activity of basolateral Na-α-KG cotransport has been shown to change organic anion transport (28, 49). However, it is unlikely that the decrease in ES uptake that followed the inhibition of tyrosine kinase or PI3K in the present study is the result of a decrease in Na-α-KG cotransport as shown by the fact that inhibition of tyrosine kinase or PI3K has no effect on basolateral transport of glutarate, a substrate for NaDC3, in rabbit renal proximal tubules (17). These previous results suggest that the effect of genistein or wortmannin on OAT3 activity in the present study reflects an action on OAT3 itself rather than on Na-α-KG cotransport or Na-K-ATPase activity.

In a previous study, we showed that the activity of OAT3 was upregulated by EGF acting via MAPK activation followed by PKA activation (41). Tyrosine kinase and PI3K have been reported to mediate EGF signaling in cell growth and differentiation of renal cells (7, 15, 16, 52) and we thought that this might be true for stimulation of OAT3 activity by EGF in isolated rabbit renal proximal tubules. Indeed, in the present study, we found that the stimulating effect of EGF on OAT3 apparently was also mediated via tyrosine kinase and PI3K activity. We also examined the relationship of tyrosine kinase and PI3K to the MAPK pathway in the regulation of OAT3 activity by inhibiting MEK, tyrosine kinase, and PI3K singly and together. The lack of an additive inhibitory effect on OAT3 activity when MEK and tyrosine kinase or MEK and PI3K were inhibited together (Fig. 4) indicates that the effects of tyrosine kinase and PI3K are part of the MAPK pathway. Because the dose of inhibitor used for each of these steps was only sufficient to reduce OAT3 activity by about 30%, a submaximum effect (Fig. 1A and Soodvaili S, unpublished observations), the lack of an additive effect could not have been the result of complete inhibition of OAT3 by one of the inhibitors.

Because our data indicated that both tyrosine kinase and PI3K were part of the MAPK pathway in the EGF-induced upregulation of OAT3-mediated organic anion transport, we attempted to determine the point in the MAPK pathway, relative to ERK, at which they acted. We observed that inhibition of PI3K led to elimination of EGF-induced ERK activation (Fig. 5), indicating that PI3K is upstream of ERK in the EGF activation of the MAPK pathway in these intact, isolated renal tubules. These results are consistent with an earlier study showing that preincubation of renal proximal tubular cells in culture with LY-294002, a PI3K inhibitor, prevented ERK activation by IGF-1 (37). However, we also observed that EGF-induced ERK activation was not attenuated by genistein in intact renal proximal tubules (Fig. 5). Therefore, it seems likely that EGF-stimulated genistein-sensitive tyrosine kinases interact with the MAPK pathway beyond ERK activation. This finding appears to differ from other findings with a number of isolated cells systems. For example, it appears that, in many systems, after ligand binding, the EGF receptor undergoes tyrosine autophosphorylation, leading to recruitment of non-receptor tyrosine kinases, followed by activation of Ras that, in turn, activates the MAPK pathway (7). In addition, experiments on human embryonic kidney-293 cells indicate that EGF-induced ERK activation is greatly reduced by genistein (18). The explanation for the difference of our findings from these previous data is not clear. However, the differences could involve the tissue model used: intact renal proximal tubules in our study vs. cultured cells in previous studies. There may be differences in response to genistein and perhaps in the intracellular signaling mechanism between the cultured cell systems and intact tissue.

From previous information on the regulation of organic anion transport by EGF (32, 33, 35, 41) and the results in the current study, we propose the model of EGF-regulated organic anion transport by OAT3 shown in Fig. 6. EGF binds to its receptor leading to activation of PI3K and some form of tyrosine kinase. Activation of PI3K leads to activation of MEK, ERK1/2, and PLÁ2. These steps lead, in turn, to an increased production of arachidonic acid, which is metabolized to PGE2 via COX1. PGE2 activates production of cAMP leading to activation of PKA and a subsequent stimulation of OAT3. EGF-stimulated genistein-sensitive tyrosine kinases interact with the MAPK pathway beyond ERK activation. The precise mechanism of interaction between genistein-sensitive tyrosine kinase and the MAPK pathway in these intact renal proximal tubules is still unclear, as is the mechanistic basis of the effect of these regulatory processes on the OAT3 protein, itself. Interestingly, the response of OAT1 activity to activation of PI3K was not changed by wortmannin on OAT3 activity in the present study reflects an action on OAT3 itself rather than on Na-α-KG cotransport or Na-K-ATPase activity.
of PKC, i.e., a marked down regulation of transport (19, 39), has been shown to arise from a rapid internalization of transport protein that does not involve phosphorylation of consensus PKC sites in the sequence of OAT1 (50). It is not known, however, whether the basis of the upregulation of OAT1 activity that follows activation of MAPK (32, 33) reflects insertion of OAT1 protein into the membrane, but it is tempting to hypothesize that this is the case. With that in mind, the similarity between the profiles of response of OAT3 and OAT1 to activation of PKC (19, 39, 41) and EGF (32, 41) makes it reasonable to hypothesize that the effects of tyrosine kinase and MAPK pathways on OAT3 activity observed in the present study are likely to reflect modulation of OAT3 trafficking in renal proximal cells and may not involve phosphorylation of the transporter itself. Finally, it seems unlikely that the stimulatory effect of EGF involves an increase in NaDC activity in light of studies showing that dicarboxylate uptake into isolated rabbit tubules does not change following inhibition of the MAPK pathway (17). However, these hypotheses need to be further investigated.

In conclusion, we present evidence that tyrosine kinase and PI3K play an important role in short-term regulation of OAT3 activity in intact rabbit renal proximal tubules. These kinases are apparently involved in the MAPK pathway for EGF stimulation of organic anion transport by OAT3. The regulation of OAT3 activity may influence the total profile of renal endogenous organic anion transport in intact tubules and thereby influence renal clearance of both toxic and therapeutic xenobiotics. Manipulation of this process may be a way of increasing therapeutic efficacy or decreasing toxicity.

GRANTS

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