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Evidence for Differential Regulation of Renal Proximal Tubular *p*-Aminohippurate and Sodium-Dependent Dicarboxylate Transport[1]

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ABSTRACT

In renal proximal tubules, the basolateral organic anion [*p*-aminohippurate (PAH)] transporter is functionally coupled to the sodium-dependent dicarboxylate transporter. This study was undertaken to elucidate whether protein kinases differentially modulate the activities of these transporters. In isolated S_2 segments of proximal tubules microdissected from rabbit kidneys, we investigated whether the transporters are regulated by tyrosine kinases, phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK). The tubules were collapsed; hence, tubular uptake of the marker substances [^{3}H]PAH and [^{14}C]glutarate reflects transport across the basolateral cell membrane. Genistein, a selective inhibitor of tyrosine kinase, diminished PAH uptake at 10^{-7} M by 15.6 ± 11.7% and at 10^{-6} M by 25.6 ± 9.1%. An inactive analog of genistein, diadzein, was without effect even at a concentration 100-fold higher than the lowest concentration of genistein, which produced significant reduction of PAH uptake. At 10^{-7} M, wortmannin, a selective inhibitor of PI3K, reduced PAH uptake by 24.1 ± 11.3% and, at 10^{-6} M, it reduced it by 32.9 ± 11.8%. The selective inhibitor of MAPK, PD98059, diminished PAH uptake at 5×10^{-5} M by 23.2 ± 6.8% and at 10^{-4} M by 18.3 ± 5.2%. Glutarate uptake was not reduced by any of these protein kinase inhibitors. Insulin had no effect on PAH uptake. These findings indicate that, in addition to protein kinase A, protein kinase C and calcium/calmodulin-dependent protein kinase II (former studies from this laboratory), as well as tyrosine kinases, PI3K, and MAPK, modulate renal basolateral PAH transport, whereas none of these protein kinases affects basolateral glutarate transport. Thus, the results provide evidence for differential regulation of basolateral transporters for PAH and dicarboxylates.

A tertiary active process has been demonstrated to mediate the transport of organic anions in proximal tubules of the kidney. A sodium/potassium ATPase establishes an inwardly directed sodium gradient that allows sodium and dicarboxylates to enter the tubular cell via the sodium-dependent dicarboxylate cotransporter. Basolateral organic anion uptake into the cell involves an exchange process with intracellularly stored dicarboxylates, namely α-ketoglutarate and glutarate (Shimada et al., 1987). The kinetic data of the sodium-dependent dicarboxylate transporter and the organic anion transporter [*p*-aminohippurate (PAH) transporter] show that the transport capacity of the dicarboxylate transporter substantially exceeds that of the PAH transporter (Stärk et al., 1997; Röver et al., 1998). Thus, at any time, intracellularly there is enough substrate for the PAH transporter to be exchanged with organic anions at the interstitial membrane of the cell.

Previous studies from this laboratory demonstrated the influence of protein kinases on transport of organic anions across the basolateral membranes of freshly isolated S_2 segments of rabbit renal proximal tubules (Hohage et al., 1994; Stärk et al., 1997; Gabriëls et al., 1998; Röver et al., 1998). The protein kinases A (PKA) and C (PKC) as well as the calcium/calmodulin-dependent protein kinase II have been shown to be involved in the regulation of the renal basolateral PAH transporter. Activation of PKC by the phorbol ester phorbol 12-myristate 13-acetate increased steady-state tubular uptake of PAH. An analysis of the kinetics of the uptake process revealed that phorbol 12-myristate 13-acetate decreased the affinity of PAH for the PAH transporter and enhanced its maximum transport capacity. Activation of PKA by dibutyryl-cyclic AMP or by a forskolin-induced increase in the intracellular cyclic AMP concentration exerted opposite effects on PKC (Hohage et al., 1994; Stärk et al.,

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ABBREVIATIONS: PAH, *p*-aminohippurate; PKA, protein kinase A; PKC, protein kinase C; TRK, tyrosine kinase; PI3K, phosphatidylinositol-3 kinase; MAPK, mitogen-activated protein kinase; MEKK, MAPKK kinase; OAT, organic anion transporter; NaDC-1, renal sodium-dicarboxylate cotransporter.

1997). Elevation as well as decrease of the intracellular calcium concentration inhibited the activity of the transporter (Gabriëls et al., 1998). The basolateral sodium-dependent dicarboxylate transporter, however, was not affected by activation of PKC and PKA or by modulation of the intracellular calcium concentration (Gabriëls et al., 1998; Röver et al., 1998).

Because those former studies indicated that the basolateral sodium-dicarboxylate cotransporter and the transporter for organic anions might be regulated differentially, in the present work, radioligand uptake studies on additional kinases were performed to provide further evidence for this assumption. In renal tubular cells as well, tyrosine kinase (TRK; Chu et al., 1996), phosphatidylinositol-3-kinase (PI3K; Derman et al., 1995; Li et al., 1995), and mitogen-activated protein kinase (MAPK; Terada et al., 1995; Chatterjee et al., 1996) have been shown to modulate cellular functions. To assess a role of these protein kinases in basolateral PAH and dicarboxylate transport, we used specific inhibitors of these kinases.

As substrates of the transporters under investigation we used [^{3}H]PAH or [^{14}C]glutarate. To examine basolateral transport most closely related to initial transport rates, short-time (30-s) uptake measurements were performed. As in our previous studies, we used nonperfused proximal S_2 segments microdissected from rabbit kidneys in which the organic anion transporter has been shown to be expressed most heavily (Sekine et al., 1997).

The results indicate that, in addition to PKA, PKC, and CaM kinase II, TRK, PI3K, and MAPK are potent regulators of the renal basolateral PAH transporter. The renal basolateral dicarboxylate transporter, however, is not affected by these kinases.

Materials and Methods

Tissue Preparation and Incubation Conditions. The methods used in this study are, in general, the same as described before (Brändle and Greven 1991a,b, 1992; Kutzer et al., 1996). In brief, male domestic rabbits, weighing 1.5 to 2.1 kg, were sacrificed by cervical dislocation. The use of animals of this weight was necessary to obtain tubules large enough for sufficient uptake of the radiolabeled compounds. A total of 130 animals were used. In each experiment, up to 15 segments of proximal tubules were microdissected from each kidney. Only superficial S_2 segments were used because these segments exhibit the highest density of PAH transporters (Sekine et al., 1997). After the dissection, the tubules were transferred in a droplet of dissection solution to an incubation device that consisted of two chambers (chambers A and B). The incubation device was placed and heated on an inverted microscope (model IM 35; Zeiss, Oberkochen, Germany). Each chamber contained 0.27 ml of incubation medium (for composition, see Kutzer et al., 1996). To replace water lost by evaporation, 30 μl of deionized water was added to the incubation medium every 10 min. This time interval was chosen to limit changes in osmolality to less than 10%.

All tubules first were incubated in chamber A and observed at a $\times$120-fold magnification to ensure that they were totally collapsed and that they were undamaged. For control measurements, individual tubules (about the half of the total amount) were subsequently transferred with a glass needle to chamber B, which contained the radioactive compounds ([^{3}H]PAH or [^{14}C]glutarate). Each tubule was incubated in chamber B for 30 s. Afterward, the agents under investigation were added to both chambers. After an incubation time of 10 min, the remaining tubules in chamber A were subsequently transferred to chamber B and again incubated for 30 s. The transfer of all tubules was accomplished after 20 to 30 min. By this maneuver, the tubules microdissected from the kidneys of one animal could be studied with control and experimental conditions. To measure the radioactivity taken up by the tubules, each tubule was taken out of chamber B separately. The radioactivity was counted for 10 min in a Beckman liquid scintillation counter (LS 6000 IC; Beckman, Fullerton, CA).

Solutions and Chemicals. The composition of the solutions was the same as in our previous studies (Kutzer et al., 1996). In the PAH uptake studies, the incubation medium also contained glutarate at a concentration of 10^{-5} M, which in our previous study was found to improve cellular [^{3}H]PAH uptake (Bartel et al., 1993). The osmolality of both solutions was adjusted to a final value of 290 mOsM/kg H_2O, and the pH was adjusted to a final value of 7.4 by adding 1 N NaOH or 1 N HCl after bubbling with 95% O_2 and 5% CO_2.

To study the cellular uptake of PAH, we added [^{3}H]PAH to the bath solution of chamber B at a concentration of 1.73 μM. This PAH concentration was lower by a factor of 17 than the minimum PAH concentration (30 μM) observed in our previous study (Bartel et al., 1993) to induce fluid secretion into and, hence, opening of the tubule lumen. To study the cellular uptake of glutarate, [^{14}C]glutarate was added at a concentration of 250 μM.

Materials. The following drugs were used in this study: *p*-[glycyl-2-^{3}H]aminohippuric acid (specific activity 5 Ci/mmol; DuPont, Dreieich, Germany); [1,5-^{14}C]glutaric acid (specific activity 20 mCi/mmol; ICN, Meckenheim, Germany); PAH, glutarate, and insulin (Sigma, Deisenhofen, Germany); genistein, diadzein, wortmannin, and PD98059 (Research Biochemicals, Incorporated, Natick, MA). All other chemicals were purchased from standard sources at the highest purity available.

Calculations. Because the tubular lumen was collapsed entirely, and assuming that the tubules were true cylinders, the following formula could be used to calculate the tubular volume (V): $V = r^2 \times \pi \times 1$, where r is the radius of the tubule and l is length. The tissue water volume was calculated by multiplying the tubular volume by a factor of 0.7 (Barfuss and Schäfer, 1979).

Because all the tubules were totally collapsed during the incubation period, we regard the PAH or glutarate uptake of each tubule as representing the quantity transported across the basolateral membrane.

Cellular [^{14}C]glutarate and [^{3}H]PAH uptake was expressed as the [^{3}H]PAH or [^{14}C]glutarate cell-to-bath ratio (related to the cell water) and was calculated as follows: cell-to-bath ratio = cellular ^{3}H or ^{14}C activity per unit volume of tissue/bath ^{3}H or ^{14}C activity per unit volume of bath.

The amount of [^{3}H]PAH or [^{14}C]glutarate taken up by unit cell volume was calculated by multiplying the [^{3}H]PAH or [^{14}C]glutarate cell-to-bath ratio by the [^{3}H]PAH or [^{14}C]glutarate bath concentration.

Statistics. Statistical treatment was carried out with the number of animals. Values are presented as means $\pm$ S.E.M. The statistical significance of differences was determined by Student's t test for paired observations.

Results

TRKs regulate several processes in renal proximal tubular cells. To test their impact on short-time organic anion uptake, in a first set of experiments, the effect of genistein, a potent and selective inhibitor of nonreceptor TRKs (Abler et al., 1992), on 30-s PAH uptake of isolated, nonperfused proximal S_2 segments was tested. For each concentration of genistein and for diadzein, its inactive analog, control measurements were performed as was done for all further substances. To be able to compare the uptake rates of different clusters of experiments, the results are depicted in percent-

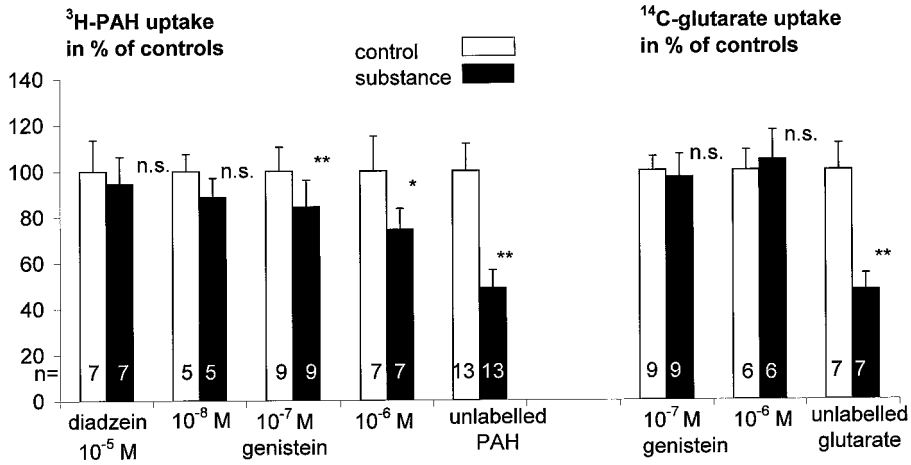

Fig. 1. Effect of genistein, inhibitor of TRKs, and its inactive analog, diadzein, on 30-s PAH and glutarate uptake into renal S_2 proximal tubules. *n*, number of animals; $^{*}P < .05$; $^{**}P < .01$; n.s., not significant.

age of controls. As shown in Fig. 1, PAH uptake was inhibited significantly by genistein. The tubules were preincubated with genistein and all further substances for 10 min. The smallest effective dose of genistein was 10^{-7} M (reduction by 15.63 ± 11.67%). Increasing the dose up to 10^{-6} M led to a further decrease of PAH uptake (reduction by 25.56 ± 9.15%). Diadzein, a structural analog of genistein that has minimal effects on protein TRKs (Shuba et al., 1996), was used as a negative control for genistein. As can be deduced from Fig. 1, diadzein (10^{-5} M) was without effect even at a concentration 100-fold higher than the lowest concentration of genistein, which produced a significant reduction of PAH uptake, whereas unlabeled PAH (10^{-3} M) reduced organic anion uptake by 51.16 ± 7.91%.

PAH is taken up into proximal tubular cells by exchange with intracellular α-ketoglutarate or glutarate. These dicarboxylates are transported into the cells by a sodium-dependent dicarboxylate transport system (Shimada et al., 1987), which also has been found to be present in the basolateral membrane of proximal S_2 segments of rabbit kidneys (Kutzer et al., 1996). To test whether the effect of genistein on PAH uptake is secondary to diminished dicarboxylate transport, we measured the effect of genistein on tubular [^{14}C]glutarate uptake. [^{14}C]Glutarate was chosen instead of α-ketoglutarate because it is not metabolized by renal cortical tissue within the time period that our experiments lasted (Pritchard, 1990). Figure 1 shows that [^{14}C]glutarate uptake was not affected significantly by genistein at the doses effective on PAH uptake whereas unlabeled glutarate (5×10^{-3} M) reduced dicarboxylate uptake by 52.26 ± 7.17%.

Figure 2 summarizes the effect of wortmannin on 30-s renal proximal tubular organic anion transport. Wortmannin, a cell-permeable fungal metabolite, binds covalently to the 110-kDa subunit of the PI3K and has been shown to inhibit PI3K, which is known to be involved in membrane-trafficking events, when added at a nanomolar concentration to mammalian cells (Kapeller and Cantley, 1994; Ui et al., 1995). The smallest effective dose of wortmannin was 10^{-7} M. At this concentration, PAH uptake was reduced by wortmannin by 24.09 ± 11.29%, and at 10^{-6} M, it was reduced by 32.90 ± 11.76%. In the case of wortmannin, basolateral glutarate uptake also was not changed in concentrations that were effective on PAH uptake.

MAPK has been examined in the opossum kidney cell line, which is a useful model of the renal proximal tubule (Terada et al., 1995), and in human proximal tubular kidney cells (Chatterjee et al., 1996). PD98059 is a selective, cell-permeable inhibitor of MAPKK kinase (MEKK), extracellular signal-regulated kinase, and MAPK/extracellular signal-regulated kinase kinase. It has been shown to inhibit activation of MAPK and the subsequent phosphorylation of MAPK substrates at high doses (Alessi et al., 1995; Dudley et al., 1995; Waters et al., 1995). Inhibition appears to be due to binding of the drug to MEKK at a site that blocks access to activating enzymes. It does not inhibit the activity of activated, i.e., phosphorylated, MEKK. As is shown in Fig. 3, at 5×10^{-5} M,

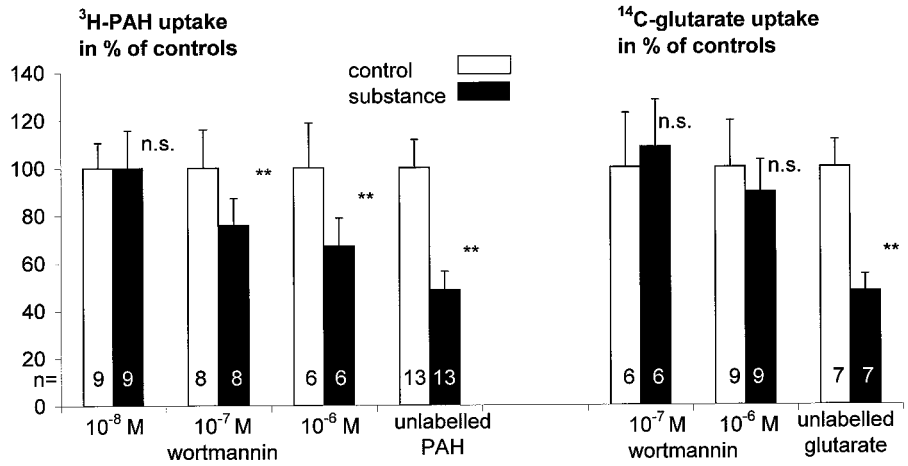

Fig. 2. Effect of wortmannin, inhibitor of PI3K, on 30-s PAH and glutarate uptake into renal S_2 proximal tubules. *n*, number of animals; $^{**}P < .01$; n.s., not significant.

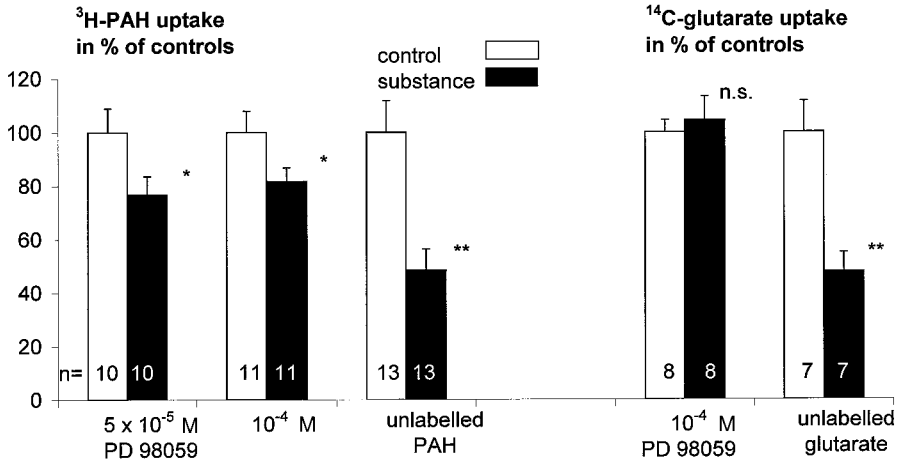

Fig. 3. Effect of PD98059, inhibitor of MAPK, on 30-s PAH and glutarate uptake into renal S_2 proximal tubules. *n*, number of animals; $^{*}P < .05$; $^{**}P < .01$; n.s., not significant.

PD98059 diminished uptake of PAH by 23.22 ± 6.78%, and at 10^{-4} M, it diminished uptake by 18.35 ± 5.17%. At 10^{-4} M, PD98059 was not effective in reducing the dicarboxylate uptake.

Insulin exerts long- and short-term regulation of the Na^+-K^+-ATPase that provides the driving force for solutes, amino acids, sugar, phosphate transport (Ewart et al., 1995), renal basolateral sodium-dependent dicarboxylate cotransport, and organic anion exchange (Shimada et al., 1987). The present results reveal that TRKs, PI3K, and MAPK have a role in the regulation of renal basolateral proximal tubular organic anion transport. Because these kinases are included in pathways that are used in cell signaling after activation of the insulin receptor (Roth et al., 1992; White and Kahn, 1994; Moule et al., 1997; Scrimgeour et al., 1997), and because of the fact that up to now no extracellular messenger consistently has been detected to modulate tubular basolateral organic anion exchange, we examined the influence of insulin on 30-s PAH uptake. As is depicted in Fig. 4, insulin did not have a significant effect on renal basolateral PAH transport (increase by 10.16 ± 6.41%).

Discussion

A wide range of organisms' own substances, xenobiotics, and drugs are secreted and reabsorbed by the proximal tubule of the kidney. Most of these substances are organic anions and cations, the transport of which has been examined, among others, in radioactive uptake studies (Boom et al., 1992; Brändle and Greven, 1992; Hohage et al., 1996). Regulation of renal basolateral organic anion transport by a protein kinase was reported first by us for PKC (Hohage et al., 1994). Activators of PKC, such as phorbol esters and diacylglycerol analogs, stimulated organic anion transport in the rabbit proximal tubule time- and dose-dependently, whereas the PAH transporter of OK cells is inhibited by this kinase (Takano et al., 1996). The stimulation of the rabbit organic anion transport was inhibited by the PKC inhibitor staurosporine (Hohage et al., 1994). Stimulation of PKA by forskolin was shown to inhibit tubular PAH uptake (Hohage et al., 1994). Calcium and calcium-dependent protein kinase II have a biphasic effect on basolateral organic anion transport. High as well as low intracellular calcium levels inhibit uptake of PAH (Gabriëls et al., 1998).

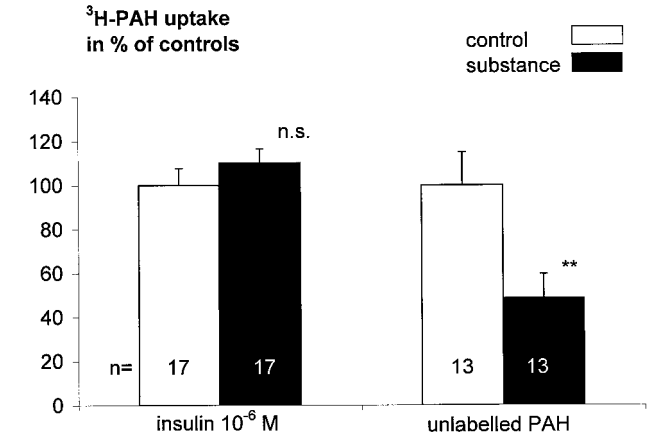

Fig. 4. Effect of insulin on 30-s PAH uptake into proximal S_2 segments. *n*, number of animals; n.s., not significant. $^{**}P < .01$.

Because we could demonstrate in previous studies that the basolateral sodium-dependent dicarboxylate transporter that uses the inwardly directed sodium gradient to provide the intracellular dicarboxylate level necessary for exchange with interstitial organic anions is not regulated by PKC, PKA, and calcium-calmodulin kinase II (Gabriëls et al., 1998; Röver et al., 1998), we tested further regulatory pathways to learn whether there is evidence for differential regulation of the coupled anion transporters. We used uptake studies of radiolabeled organic anions into freshly isolated S_2 segments of rabbit renal proximal tubules, the lumen of which was collapsed. The advantage of using intact tubules with collapsed lumen instead of isolated cells is that the substance taken up via the basolateral membrane does not leak out of the cell laterally or into the lumen in this model. Thus, the radioactivity counted reflects the total amount taken up basolaterally.

Membrane transport and secretion are regulated by the phosphorylation of serine, threonine, and tyrosine residues; this phosphorylation triggers conformational changes in regulated proteins. Pajor (1996) cloned a rabbit and a human renal sodium-dicarboxylate cotransporter (NaDC-1 and hNaDC-1). In the human but not in the rabbit transporter, she identified two potential PKC phosphorylation sites. The PAH transporter has been cloned by three groups from different species. Several potential modification sites were detected. Sekine et al. (1997), who isolated the rat sodium-

dependent dicarboxylate cotransporter (rNaDC-1) and the organic anion transporter 1 from rat kidney (OAT1), did not comment on phosphorylation sites of the rat NaDC-1 but found four putative PKC-dependent phosphorylation sites in the hydrophilic loop between transmembrane domains 6 and 7 of the OAT1. Sweet et al. (1997) identified a possible PKC site at a large, extracellular loop of an organic anion transport protein from rat kidney (ROAT1) as well as four more PKC consensus sites and three potential casein kinase II sites, which may or may not be located intracellularly, depending on the modeling program used. Reid et al. (1998), who cloned a human PAH transporter, detected four potential phosphorylation sites for PKC and consensus sites for casein kinase II-dependent phosphorylation in a large, cytoplasmic loop between transmembrane domains 6 and 7 as well as further sites for PKC, casein kinase II, and TRKs in the C terminus. Yet, sequence and consensus sites of a rabbit PAH transporter have not been reported.

Involvement of Tyrosine Phosphorylation. In the present study we used genistein as a probe to establish a role of tyrosine protein kinases in the regulation of the renal basolateral PAH transporter. The use of membrane-permeant, selective inhibitors as probes to examine a functional role of an intracellular-signaling protein is of particular advantage because it is not necessary to disturb the integrity of the cell by applying these tools. Genistein inhibits protein TRKs but is far less active against other known kinases. However, it may also have effects unrelated to inhibition of TRKs (Shuba et al., 1996). The isoflavon agent has been reported to be a competitive inhibitor of ATP binding to the catalytic domain of TRKs, which may be the reason for the lack of an inhibitory effect on receptor TRK activity of the insulin receptor. The insulin receptor, in contrast to the monomeric structure of other receptors, forms heterodimers that hinder the access of genistein to the ATP-binding site on the insulin receptor (Abler et al., 1992). In our study, the lowest concentration of genistein that significantly inhibited tubular PAH transport was 10^{-7} M. It seems unlikely that the inhibition of PAH transport by genistein was due to a nonspecific effect of the inhibitor because diadzein, a structural analog of genistein that has little inhibitory effect on protein TRKs, failed to inhibit anion transport at 10^{-5} M. Furthermore, that diadzein did not affect organic anion uptake renders it unlikely that the inhibitory effect of genistein on PAH uptake was mediated by direct inhibition of the PAH transporter. In a study by Good (1995), genistein has been shown to block the inhibition of the thick ascending limb Na^+/H^+ antiporter by hyperosmolarity. Furthermore, TRK pathways contribute to the activation of the renal proximal tubule apical membrane Na^+/H^+ antiporter by endothelin B receptors (Chu et al., 1996). In addition to these findings, we now demonstrated that PAH uptake is aided by TRKs.

Because it is well established that the tubular uptake of PAH across the basolateral membrane involves an exchange process with intracellular dicarboxylates, namely α-ketoglutarate or glutarate, which are transported by the basolateral sodium-dependent dicarboxylate transporter into the cells (Shimada et al., 1987), the effect of genistein on tubular PAH uptake might be due to a genistein-induced inhibition of the basolateral dicarboxylate transporter, thus decreasing the cell-to-bath concentration gradient of these dicarboxylates that drives PAH into the cells. However, genistein did not, even at the concentration of 10^{-6} M, affect the dicarboxylate transporter. Thus, inhibition of TRK activity may directly affect the PAH transporter either by decreasing the number of PAH-binding sites or by altering the coupling coefficient of the PAH/dicarboxylate exchange reaction.

Involvement of PI3K. Several types of PI3K recently have been cloned. Most studied is the heterodimeric PI3K, which consists of a regulatory 85-kDa subunit and a 110-kDa subunit. Two genes for each subunit have been identified (von Willebrand et al., 1996). Wortmannin has been proven to be a potent inhibitor of mammalian PI3K in virtually all preparations tested so far. With purified enzymes and cells, an IC_{50} of ~3 nM was found (Ui et al., 1995) whereas in intact tissues, higher concentrations have been demonstrated to be necessary (Ito et al., 1997; Sheperd et al., 1997; Zheng et al., 1997). We used this cell-permeable fungal metabolite as a tool to demonstrate a role of PI3K in the regulation of the renal organic anion transporter. In the present study, wortmannin significantly inhibited tubular PAH transport at a very low concentration (10^{-7} M), which renders it very unlikely that unspecific effects cause this result.

Involvement of MAPK. MAPK appears to be expressed generally in all cell types examined to date, but the physiological role of this protein kinase in renal epithelial cells is far from deciphered. MAPK has been shown to be active in cortical (Wong et al., 1995), inner medullary collecting duct (Heasley et al., 1994), and proximal tubular cells (Terada et al., 1995; Chatterjee et al., 1996). PD98059, the inhibitor of MAPK, which has been demonstrated not to affect the activities of 18 different serine/threonine kinases, four different TRKs, and the PI3K at the concentration used in our studies (Alessi et al., 1995), clearly reduced 30-s organic anion uptake after 10 min of incubation. In contrast to this finding, PI3K was not effective, even at the concentration of 10^{-4} M, in modulating dicarboxylate transport. Thus, inhibition of the MAPK cascade seems to affect the PAH transporter but not the sodium/glutarate cotransporter.

MAP kinase and the network of protein kinases involved in their regulation are activated by receptor TRKs as well as G protein-coupled receptors. Furthermore, the MAPK pathway is stimulated after direct activation of PKC with phorbol esters in many cell types. Thus, MAPK appears to be a convergence point for multiple intracellular signaling pathways (Heasley et al., 1994).

Involvement of the Insulin Receptor. Insulin is known to regulate both metabolic and transport functions in the renal proximal tubule. It stimulates amiloride-sensitive sodium transport in A6 cells by additive mechanisms (Record et al., 1996), increases Na^+-H^+ exchange activity in proximal tubules from normotensive and hypertensive rats (Gesek and Schoolwerth, 1991), and enhances sodium sensitivity of Na^+-K^+-ATPase in isolated rat proximal convoluted tubule (Féraille et al., 1994). Insulin activates rapidly a complex cascade of protein kinases, leading to stimulation of mitogenic and metabolic events. In the present investigation of 30-s organic anion uptake, a significant effect of insulin on PAH transport could not be shown, although we demonstrated TRKs, PI3 kinase, and MAP kinase, which are used in cell signaling after activation of the insulin receptor to have a role in the regulation of renal basolateral proximal tubular organic anion transport. These kinases have been shown to be activated by other extracellular signals as well.

Clearly, further studies are needed to elucidate at least three questions: 1) the mechanism of action of the kinases discussed above in the basolateral PAH transport; 2) which of the multiple potential sites of actions on the transporter are involved in this effect; and 3) the interdependence of the kinases active in this process.

Taken together, the data presented herein indicate that TRKs, PI3K, and MAPK have a tonic stimulatory effect on renal basolateral PAH transport, whereas there is no effect of these protein kinases on renal basolateral glutarate transport. The results provide evidence for differential regulation of the basolateral transporters for PAH and dicarboxylates.

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