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Transport of *N*-Acetylcysteine *S*-Conjugates of Methylmercury in Madin-Darby Canine Kidney Cells Stably Transfected with Human Isoform of Organic Anion Transporter 1

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ABSTRACT

Recent studies have implicated the activity of the organic anion transporter 1 (OAT1) protein in the basolateral uptake of inorganic mercuric species in renal proximal tubular epithelial cells. However, very little is known about the potential role of OAT1 (and other OATs) in the renal epithelial transport of organic forms of mercury such as methylmercury (CH_3Hg^+). The present investigation was designed to study the transport of *N*-acetyl cysteine (NAC) *S*-conjugates of both methylmercury (CH_3Hg-NAC) and inorganic mercury (NAC-Hg-NAC) in renal epithelial cells [Madin-Darby canine kidney (MDCK) cells] stably transfected with the human isoform of OAT1 (hOAT1). These mercuric species were studied because numerous mercapturates have been shown to be substrates of OATs. Data on saturation kinetics, time dependence, substrate specificity, and temperature dependence for the transport of CH_3Hg-NAC and NAC-Hg-NAC indicate that both of these two mercuric species are indeed transportable substrates of hOAT1. Substrate specificity data also show that CH_3Hg-NAC is a substrate of a transporter in MDCK cells that is not hOAT1. These data indicate that an amino acid carrier system is a likely candidate responsible for this transport. Furthermore, the rates of survival of the hOAT1-transfected MDCK cells were significantly lower than those of corresponding control MDCK cells when they were exposed to cytotoxic concentrations of CH_3Hg-NAC or NAC-Hg-NAC. Collectively, the present data support the hypothesis that CH_3Hg-NAC and NAC-Hg-NAC are transportable substrates of OAT1 and thus potentially transportable mercuric species taken up in vivo at the basolateral membrane of proximal tubular epithelial cells.

Once mercuric ions enter into systemic circulation following exposure to inorganic or organic forms of mercury, they have a propensity to accumulate preferentially in the epithelial cells lining the proximal segments of the nephron, particularly in the pars recta of proximal tubules (Zalups and Lash, 1994; Zalups, 2000b). The mechanisms by which these metal ions gain entry into the target epithelial cells in the kidneys are not currently fully elucidated. However, there is evidence indicating that when mercuric ions bind to reduced sulfur atoms of various endogenous nonprotein thiols, the organo-metal complexes formed have the ability to behave as molecular "mimics" or functional homologs of essential biomolecules (such as amino acids and organic acids) at the sites of membrane transporters involved in the uptake of these biomolecules (Clarkson, 1993; Simmons-Willis et al., 2002; Zalups and Ahmad, 2004; Zalups et al., 2004). For example, recent cellular and molecular evidence from our laboratory indicates that the linear II, coordinate-covalent mercuric conjugate of cysteine (Cys), Cys-Hg-Cys, can serve as a molecular mimic of the amino acid cystine (Cys-*S*-*S*-Cys) at the site of the luminal amino acid transporter system $b^{0,+}$ (Cannon et al., 2000; Zalups, 2000b; Cannon et al., 2001; Bridges et al., 2004).

It is important to stress that the uptake of inorganic mercury (Hg^{2+}) by proximal tubular epithelial cells in vivo is not restricted to the luminal plasma membrane. Numerous lines of evidence indicate that mercuric ions are also taken up from the blood and extracellular fluid at the basolateral membrane by one or more organic anion transport system(s) (Zalups, 1995, 1998a,b; Zalups and Barfuss, 1995, 1998a,b, 2002; Zalups et al., 1998). Within the kidneys, these trans-

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ABBREVIATIONS: Cys, cysteine; OAT, organic anion transporter; NAC, *N*-acetylcysteine; MDCK, Madin-Darby canine kidney; hOAT, human isoform of organic anion transporter; Hcy, homocysteine; PAH, *p*-aminohippuric acid; EMEM, Eagle's modified essential medium; HBSS, Hanks' buffered saline solution; DMPS, 2,3-dimercaptopropane-1-sulfonic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide; Cys-Hg-Cys, cysteine *S*-conjugate of inorganic mercury; CH_3Hg-NAC, *N*-acetylcysteine *S*-conjugate of methylmercury; NAC-Hg-NAC, *N*-acetylcysteine *S*-conjugate of inorganic mercury.

port systems are localized exclusively along the proximal tubule, where they provide a secretory mechanism for the elimination of many xenobiotics and endogenous organic anions that escape filtration during their passage through the kidney(s).

One of the best characterized organic anion transport systems mediates the basolateral uptake of a wide range of organic anions by a dicarboxylate/organic anion exchanger, which has been named organic anion transporter (OAT) 1. This membrane protein transports organic anions into the cytosolic compartment of proximal tubular epithelial cells in exchange for molecules of α-ketoglutarate (Pritchard and Miller, 1996). The intracellular-to-extracellular gradient of α-ketoglutarate is maintained by both intracellular metabolism and reclamation of α-ketoglutarate from the extracellular compartment by the sodium-coupled, dicarboxylate transporter present in the basolateral membrane of proximal tubular cells (Pritchard and Miller, 1996).

Although there is considerable evidence from mammalian renal epithelial cells implicating thiol *S*-conjugates of inorganic mercury as substrates of OAT1 (Aslamkhan et al., 2003b; Zalups and Ahmad, 2004; Zalups et al., 2004), little is known about the potential role of this membrane transporter in the basolateral uptake of thiol *S*-conjugates of organic forms of mercury by proximal tubular epithelial cells. Considering that methylmercury is the primary form of mercury present in the environment, it is important to understand the mechanisms involved in the transport and handling of this species of mercury in target cells affected by this toxicant.

Accordingly, the present study was designed to characterize and compare the transport of NAC *S*-conjugates of both methylmercury (CH_3Hg-NAC) and Hg^{2+} (NAC-Hg-NAC) in Madin-Darby canine kidney (MDCK) cells stably transfected with the human isoform of OAT1 (hOAT1). Considering that MDCK cells are derived from the distal nephron, direct molecular evidence for the participation of OAT1 in the transport of specific mercuric species could be obtained from a line of mammalian renal epithelial cells that do not express organic anion transporters. One rationale for first studying the transport of NAC *S*-conjugates of methylmercury is that NAC endows the CH_3Hg-NAC with a polar, negative charge, which makes the complex a potentially better substrate for OAT1 than the neutral complexes formed by the conjugation of CH_3Hg^+ with Cys or homocysteine (Hcy). Moreover, by comparing the transport of NAC *S*-conjugates of both Hg^{2+} and CH_3Hg^+, a more complete understanding can be gained about the extent to which OAT1 handles mercapturic acid forms of two very toxicologically relevant chemical species of mercury.

Data from the present study show for the first time that mercapturic acid forms of CH_3Hg^+ are indeed transportable substrates in mammalian renal epithelial cells expressing OAT1. Moreover, these data also demonstrate for the first time that CH_3Hg-NAC is a likely substrate of one or more amino acid transport systems.

Materials and Methods

Transfection of MDCK II Cells with hOAT1. A subclone of mycoplasma-free type II MDCK cells (originally provided by Dr. Daniel Balkovetz, University of Alabama, Birmingham, AL) was used in this investigation. These cells were originally developed in the laboratory of Dr. Kai Simons (European Molecular Biology Laboratory, Heidelberg, Germany). As described previously (Aslamkhan et al., 2003a,b), these cells were transfected with the cDNA for hOAT1 ligated to pcDNA3.1 (Invitrogen, Carlsbad, CA) using QIAGEN SuperFect reagent (Chatsworth, CA) according to the manufacturer's protocol (5 μl of SuperFect per microgram of DNA). Subclones of hOAT1-expressing (and wild-type) MDCK II cells used in the present investigation were provided as a gift by Dr. John Pritchard [National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health, Bethesda, MD]. These subclones were maintained in culture media with 200 μg/ml geneticin (G418; Invitrogen) and screened regularly to determine whether they had gained the ability to transport organic anions. This was accomplished by assaying the uptake of [^{3}H]*p*-aminohippurate (PAH) as described below.

Cell Culture. When wild-type and hOAT1-expressing MDCK cells were grown in a confluent monolayer, the cell-to-cell attachments afforded a low transepithelial resistance. All MDCK II cells were grown at 37°C in Eagle's minimum essential medium (EMEM) (Invitrogen) supplemented with 1 mM sodium pyruvate and 10% fetal bovine serum (Invitrogen). While in culture, the MDCK cells were grown and maintained in a humidified atmosphere consisting of 95% O_2 and 5% CO_2. Cells were split every 3 to 7 days, and 1/10 to 1/20 of the culture was inoculated into new flasks.

Uptake of PAH in hOAT1-Transfected and Nontransfected MDCK II Cells. Dr. Pritchard's laboratory had established that the insertion of hOAT1 protein occurs at both apical and basolateral plasma membranes in the MDCK cells transfected with the cDNA encoding hOAT1. Apical expression of hOAT1 permits one to study hOAT1-dependent transport using cells grown on a solid surface. Therefore, the uptake of [^{3}H]PAH (as well as other substrates) was assessed in cells plated in 24-well (2.0 cm^2/well) cell culture cluster plates (Costar Corning, New York, NY) containing supplemented EMEM at a density of 0.5×10^6 cells/well (added as 2 ml). The cells were grown in a humidified atmosphere of 95% O_2 and 5% CO_2 for 2 days at 37°C, with media being changed after the first 24 h.

Immediately prior to assessing transport parameters, each well containing cells was first rinsed with Hanks' buffered saline solution (HBSS) supplemented with 10 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (pH 7.4) for three consecutive 5-min periods. At the beginning of each experiment, 350 μl of the aforementioned Hanks' buffer containing 5.0 μM PAH, with or without of 200 μM probenecid, was added to each well. Some of the PAH was in the form of [^{3}H]PAH (4.54 mCi/μmol; PerkinElmer Life and Analytical Sciences, Boston, MA). Following 60 min of exposure to PAH, the cells in each well were rinsed with cold (4°C) "stop" buffer [HBSS supplemented with 10 mM HEPES (pH 7.4)]. To determine the cellular content of [^{3}H]PAH, cells were first lysed by adding 1 ml of 1 N NaOH to each well. The plates containing NaOH were shaken overnight (for at least 12 h) in an orbital shaker operating at a rate of 500 rpm. Subsequently, 700 μl of cellular lysate from each well were neutralized with 700 μl of 1 N HCl. The total volume of neutralized solution was added to 15 ml of Opti-Fluor high flash-point liquid scintillation fluid (PerkinElmer Life and Analytical Sciences). The radioactivity in each sample was determined using a Beckman LS6000IC Liquid Scintillation Analyzer (Beckman Coulter, Fullerton CA). The remaining cellular lysate (50 μl) from each well was used to determine the total amount of protein per well using the Bradford protein assay (Bradford, 1976). Transport data obtained from each well of cells were normalized to the corresponding concentration of cellular protein, and the rates of cellular uptake of [^{3}H]PAH were expressed as picomoles $\times$ milligram of protein^{-1} $\times$ minute^{-1}.

Experimental Design. Concentration dependence, time dependence, temperature dependence, and substrate specificity for the extracellular-to-intracellular transport of CH_3Hg-NAC and NAC-Hg-NAC were characterized and compared in both hOAT1 transfectants and corresponding wild-type control MDCK cells. To determine

whether CH_3Hg-NAC is a transportable substrate of hOAT1, concentration-response data for the uptake of CH_3Hg^+ (in the form of CH_3Hg-NAC) and Hg^{2+} (in the form of NAC-Hg-NAC) were fitted to the following Michaelis-Menten equation:

$$V = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

In this equation, V represents velocity, V_{max} is the maximal velocity for transport of the substrate studied, $[S]$ is the concentration of the substrate being transported, and K_m is the Michaelis-Menten constant. In addition, linear regression analysis was applied to transport data plotted by the Eadie-Hofstee method, where V is plotted against $V/[S]$. The slope of the regression lines determined by this method is equal to $-K_m$. By using these equations, we were able to characterize the transport of NAC *S*-conjugates of both Hg^{2+} and CH_3Hg^+.

Transport of CH_3Hg^+ and Hg^{2+} in hOAT1-Transfected and Nontransfected MDCK II Cells. In the experiments where the transport of NAC *S*-conjugates of CH_3Hg^+ and Hg^{2+} were studied, cells were also plated in 24-well (2.0 cm^2) cell culture cluster plates (Costar Corning) containing supplemented EMEM at a density of 0.5×10^6 cells/well (added as 2 ml). They were then grown in a humidified atmosphere of 95% O_2 and 5% CO_2 for 2 days at 37°C, with media being changed after the first 24 h. During the assessment of transport activity, the medium was aspirated from the wells, and cells were rinsed three times with 3 ml of HBSS supplemented with 10 mM HEPES (pH 7.4). Three-hundred fifty microliters of transport buffer (specific to each experiment) containing radioactive inorganic mercury ($^{203}Hg^{2+}$; 8–12 mCi/mg mercury) or methylmercury ($[^{14}C]H_3Hg^+$ (20 mCi/mmol); American Radiolabeled Chemicals, St. Louis, MO) was added to each well.

In selective experiments, competitive inhibitors of OAT1, such as PAH or the dicarboxylates adipate or glutarate, were added to the transport buffer. In additional experiments, the influence of various amino acids (such as leucine, isoleucine, methionine, alanine, phenylalanine, etc.) on the transport of CH_3Hg-NAC was assessed.

The concentration of CH_3Hg-NAC or NAC-Hg-NAC used in most transport experiments was 5 μM. The primary rationale for choosing this concentration is that, in a separate set of toxicity experiments, concentrations of these mercuric species at 10 μM or lower did not induce any cytotoxic effects in either the hOAT1-transfected or wild-type MDCK cells for up to 6 h.

NAC (as the L-isomer) was added in a 4:1 mol ratio with Hg^{2+} to ensure that each mercuric ion in solution formed a thermodynamically stable, linear II, coordinate-covalent complex with two molecules of NAC. On the other hand, NAC was added in a 2:1 mol ratio with CH_3Hg^+ to ensure that each methylmercuric ion in solution formed a thermodynamically stable, linear I, coordinate-covalent complex with a molecule of NAC. The association constant between mercuric ions and the sulfur atom of low molecular weight thiols is 10 orders of magnitude greater than those between mercuric ions and any other biologically occurring nucleophilic groups (Zalups, 2000b).

At the end of a particular exposure to one of the mercuric species studied, the cells in each well were rinsed with cold (4°C) stop buffer [HBSS supplemented with 10 mM HEPES (pH 7.4) containing 1 mM 2,3-dimercaptopropane-1-sulfonic acid (DMPS) and 200 μM probenecid]. DMPS is a very effective dithiol chelator of mercuric ions. It was used to reduce the pool of mercuric ions bound to the outer surfaces of the plasma membrane to negligible levels. Since DMPS oxidizes rapidly in aqueous solutions, it was mixed into solution within the first 15 min of its use. Probenecid was used in the stop buffer as an added measure to inhibit the activity of OAT1 at the termination of each experiment.

Cellular content of Hg^{2+} was determined using both γ spectrometry (Wallac Wizard 3″ 1480 Automatic Gamma Counter; PerkinElmer Wallac, Gaithersburg, MD) and liquid scintillation spectrometry (LS6000IC Liquid Scintillation Analyzer; Beckman Coulter). The cellular content of ^{203}Hg was determined by γ spectroscopy, whereas the cellular content of ^{14}C-labeled CH_3Hg^+ was determined by liquid scintillation spectrometry. When liquid scintillation counting was employed, 1 ml of 1 N NaOH was added to each well. After adding the NaOH, the 24-well plates were shaken in an orbital shaker at 500 rpm for 24 h. Cellular lysate (700 μl) from each well was neutralized with 700 μl of 1 N HCl. The total volume of neutralized solution was added to 15 ml of Opti-Fluor (PerkinElmer Life and Analytical Sciences) scintillation fluid. The radioactivity of each sample was determined using an LS6000IC Liquid Scintillation Analyzer (^{203}Hg counting efficiency, ~80–90%). The remaining cellular lysate (50 μl) from each well was used to determine the total amount of protein per well using the Bradford protein assay. Transport data obtained from each well of cells were normalized to the corresponding concentration of cellular protein.

Assessment of Toxicity and Cellular Viability. The effects of the mercuric conjugates CH_3Hg-NAC and NAC-Hg-NAC on cellular viability were measured using a methylthiazoletetrazolium (MTT)-based toxicity assay (Sigma-Aldrich, St. Louis, MO). This assay measures the activity of mitochondrial dehydrogenase by the conversion of the yellow tetrazolium dye MTT to purple formazan crystals. Cells were plated in supplemented EMEM at a density of 5.0×10^4 cells/well (added as 200 μl/well) in sterile 96-well microtiter plates (Costar Corning) and allowed to grow for 48 h in a humidified atmosphere of 5% CO_2/95% O_2 at 37°C. Supplemented EMEM was changed after the first 24 h by inversion. Excess media adhering to the plate were blotted off with sterile gauze (Johnson & Johnson, Arlington, TX). After 48 h, wells were again washed two times with 200 μl of HBSS per well. After washing, test compounds were added to individual wells (200 μl/well) in unsupplemented EMEM, and cells were grown for 6 h in a humidified atmosphere of 5% CO_2/95% O_2 at 37°C. At the conclusion of the exposure period, media were removed by inversion and blotting, wells were washed with 200 μl of HBSS, and 100 μl of 0.5 mg/ml (1.2 mM) MTT in HBSS was added to each well. Cells were incubated for 2 h, and 100 μl of solubilization buffer (10% Triton X-100, 0.1 N HCl in isopropyl alcohol) was added to each well. This buffer both lysed the cells (releasing the formazan) and dissolved the water-insoluble formazan crystals. After an overnight incubation at room temperature, full solubilization had occurred, and plates were read at 595 nm in a Titertek Multiskan MKII plate reader (Fisher Scientific Co., Suwanee, GA).

Production of $^{203}Hg^{2+}$. Three milligrams of mercuric oxide (HgO) containing the stable isotope $^{200}Hg^{2+}$ and enriched $^{202}Hg^{2+}$ (target) was weighed and doubly sealed in quartz tubing (actual mercuric oxide isotopic composition: <0.05% ^{196}Hg, 1.5% ^{198}Hg, 2.82% ^{199}Hg, 4.24% ^{200}Hg, 3.11% ^{201}Hg, 86.99% ^{202}Hg, and 1.34% ^{204}Hg). The double-encapsulated target was sent to the Missouri University Research Reactor facility to be irradiated (by neutron activation) for 4 weeks. The irradiated target was placed in protected storage for 10 days to allow for the isotopic decay of the newly formed $^{197}Hg^{2+}$. The target was removed from the quartz tubing with four 50-μl rinses of 1 N HCl. All four rinses were placed and sealed in a single 1.7-ml polypropylene vial. A sample of the solution was then used to determine the precise solid content of mercury using plasma-coupled elemental mass spectrometry. The radioactivity of the solution was determined with a Wallac Wizard 3″ 1480 Automatic Gamma Counter (^{203}Hg counting efficiency, ~50%). The specific activities of the $^{203}Hg^{2+}$ used in this study ranged between 8 and 12 mCi/mg mercury.

Statistical Analysis. Results are presented as representative data from at least two sets of experiments. Data are expressed as the mean ± S.E. For uptake studies, a sample size of $n = 3$ or 4 was used. Assuming that each sample was mutually independent, statistical analysis for each parameter assessed was performed by first using a two-way analysis of variance followed by either Tukey's or Dunnett's post hoc test. Data expressed as a percentage were first normalized using the arcsine transformation prior to applying parametric sta-

tistical analyses. This transformation takes the arcsine of the square root of the decimal fraction of the percentage score. Differences among means were considered statistically significant at $p < 0.05$.

Results

Uptake of PAH. Expression of a functional hOAT1 transporter protein was confirmed in the hOAT1-transfected MDCK II cells by assessing the uptake of [^{3}H]PAH (which is a high-affinity substrate of this organic anion/dicarboxylate exchanger). When presented to the cells at a concentration of 5 μM, PAH was taken up by the hOAT1-transfected MDCK cells at a rate of 9.36 $\pm$ 0.66 pmol $\times$ min^{-1} $\times$ mg protein^{-1}. Moreover, 200 μM probenecid decreased the rate of uptake of PAH in the transfected cells to an average of 0.75 $\pm$ 0.14 pmol $\times$ min^{-1} mg of protein^{-1}.

The uptake of PAH in the wild-type MDCK cells was insignificant, averaging only approximately 0.10 $\pm$ 0.01 pmol $\times$ min^{-1} $\times$ mg protein^{-1}. Unlike in the hOAT1-transfected cells, the addition of 200 μM probenecid to the extracellular compartment did not significantly affect the uptake of PAH in the wild-type control cells (0.10 $\pm$ 0.01 pmol $\times$ min^{-1} mg $\times$ protein^{-1}). These gain-of-function findings indicate, therefore, that a functional form of hOAT1 was indeed being inserted into the plasma membranes of the hOAT1-transfected MDCK cells.

Concentration-Dependent Uptake of CH_3Hg^+. The assessment of the concentration dependence and saturation kinetics for the uptake of CH_3Hg^+ revealed that the uptake of this form of mercury, when presented as CH_3Hg-NAC, was greater in hOAT1-transfected MDCK II cells than in corresponding control cells (Fig. 1A). Interestingly, the kinetic data indicate that carrier-mediated processes were likely involved in the uptake of CH_3Hg^+ in both the hOAT1-transfected and wild-type control MDCK II cells, although the levels of uptake were overall greater in the hOAT1-transfected cells. In the wild-type MDCK cells, the analysis of kinetic parameters (Eadee-Hofstee analysis shown in Fig. 1A inset) revealed that the apparent K_m for the uptake of CH_3Hg^+ was 180 $\pm$ 27 μM and that the apparent V_{max} was 325 $\pm$ 24 pmol $\times$ min^{-1} $\times$ mg protein^{-1}.

The kinetics for the uptake of CH_3Hg^+ in the transfected MDCK cells that could be attributed to the activity of hOAT1 (hOAT1 data minus control data) revealed that the apparent K_m for the uptake of CH_3Hg^+ was 79.5 $\pm$ 23.0 μM and that the apparent V_{max} was 278 $\pm$ 17.9 pmol $\times$ min^{-1} $\times$ mg protein^{-1} (Fig. 1B). Eadee-Hofstee analysis for this uptake is represented in the Fig. 1B inset.

Analysis of the uptake of Hg^{2+}, when presented as the mercuric conjugate NAC-Hg-NAC, indicated that there was concentration-dependent uptake of Hg^{2+} in both the hOAT1-transfected and wild-type control MDCK II cells but that the rates of uptake were significantly greater in corresponding hOAT1-transfected cells (Fig. 2). Analysis of the kinetics for the uptake of Hg^{2+} revealed that carrier-mediated uptake was likely only involved in the uptake of Hg^{2+} in the hOAT1-transfected cells. The apparent K_m and V_{max} for the uptake of Hg^{2+} in the hOAT1-transfected MDCK cells were calculated to be 144 $\pm$ 29 μM and 108 $\pm$ 10 pmol $\times$ min^{-1} $\times$ mg protein^{-1}, respectively. The association of Hg^{2+} in the control cells likely represents nonspecific binding.

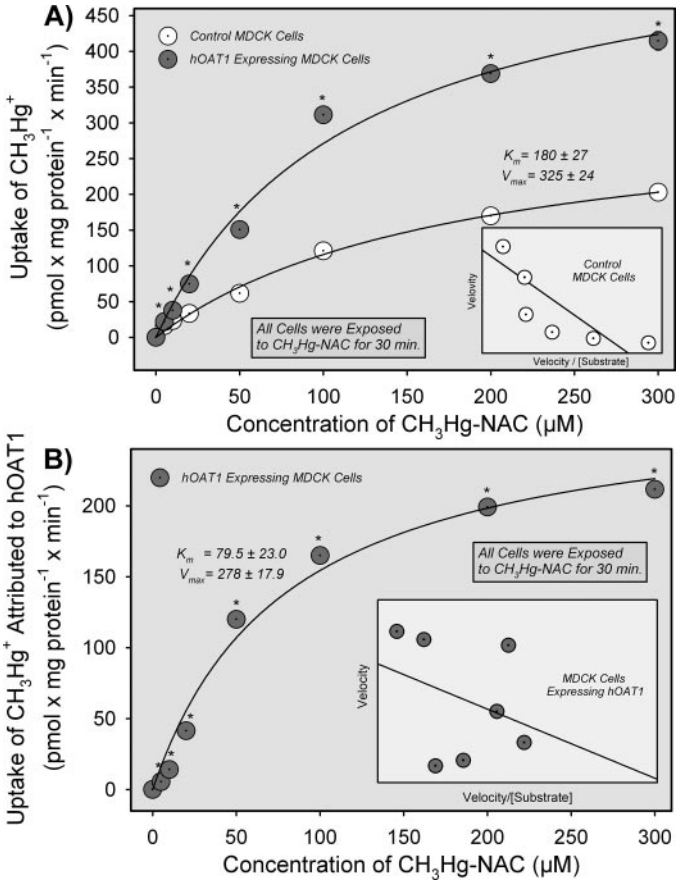

Fig. 1. A, concentration-dependent uptake (picomoles $\times$ minute^{-1} $\times$ milligram of cellular protein^{-1}) of CH_3Hg^+ in control and hOAT1-expressing MDCK II cells exposed to CH_3Hg-NAC. B, kinetic analysis of the concentration-dependent uptake of CH_3Hg^+ attributable to hOAT1. Cellular uptake was studied for 30 min (at 37°C). Values are mean $\pm$ S.E. *, significantly different ($p < 0.05$) from the mean for the corresponding group of control MDCK cells. Data for the control and hOAT1-expressing MDCK cells in A were fitted to the nonlinear regression equation $y = V_{max} \times X/(K_m + X)$ with a resulting $r^2 = 0.9969$ and an $r^2 = 0.9922$, respectively. Linear regression analysis of the Eadie-Hofstee data for the control MDCK cells in A reveals a good fit between the experimental data and the predicted line ($r^2 = 0.8332$). Data for the hOAT1 component in B fit the nonlinear regression equation $y = V_{max} \times X/(K_m + X)$ with an $r^2 = 0.9902$. The data expressed in the Eadie-Hofstee plot for the hOAT1 component in B fit a linear equation with an $r^2 = 0.8332$.

Time-Dependent Uptake of CH_3Hg^+ and Hg^{2+}. Time-dependent uptake of CH_3Hg^+, when presented as 5 μM CH_3Hg-NAC, was detected in both the hOAT1-transfected and wild-type control cells (Fig. 3). However, the rates of uptake of CH_3Hg^+ were always significantly greater in the MDCK cells transfected with the cDNA for hOAT1 than in the corresponding control MDCK cells at all times studied. The greatest differences in the transport of CH_3Hg^+ between the hOAT1-transfected and wild-type control cells were detected at 60 and 120 min of exposure.

When 5 μM NAC-Hg-NAC was present in the media, the uptake of Hg^{2+} in the hOAT1-transfected MDCK cells occurred in a time-dependent manner (Fig. 3). Transport of Hg^{2+} was also always greatest in the hOAT1-transfected cells than in the corresponding control cells. In the control MDCK cells, uptake of Hg^{2+} increased significantly only after approximately 4 h of exposure to 5 μM NAC-Hg-NAC.

Effect of PAH and Probenecid on the Uptake of CH_3Hg^+ and Hg^{2+}. The addition of 1 mM PAH or 200 μM

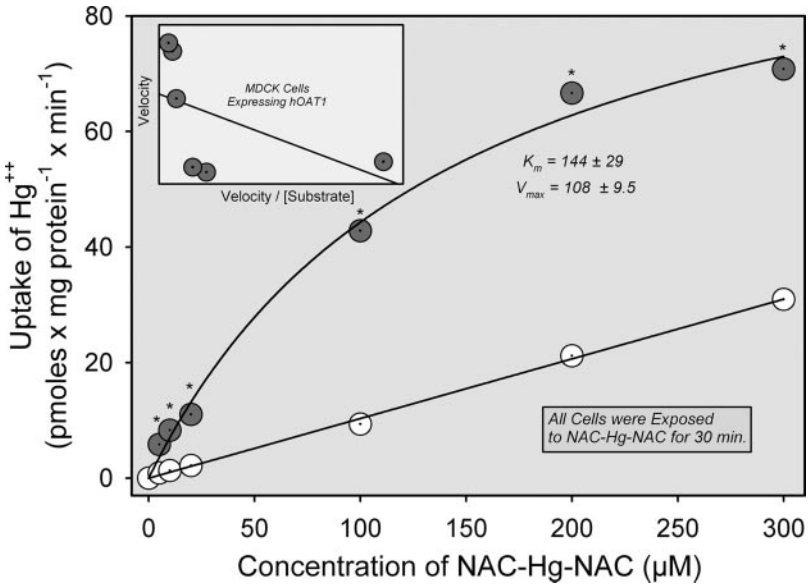

Fig. 2. Concentration-dependent uptake (picomoles × minute^{-1} × milligram of cellular protein^{-1}) of Hg^{2+} in control and hOAT1-expressing MDCK II cells exposed to NAC-Hg-NAC, respectively. Cellular uptake was studied for 30 min (at 37°C). Values are mean ± S.E. ∗, significantly different ($p < 0.05$) from the mean for the corresponding group of control MDCK cells. Data for the hOAT1-expressing MDCK cells fit the nonlinear regression equation $y = V_{max} \times X/(K_m + X)$ with an $r^2 = 0.9960$. The data for the MDCK cells were fitted to a linear equation with a resulting $r^2 = 0.9987$. The data in the Eadie-Hofstee plot for the control MDCK cells in A fit a linear equation with an $r^2 = 0.5762$.

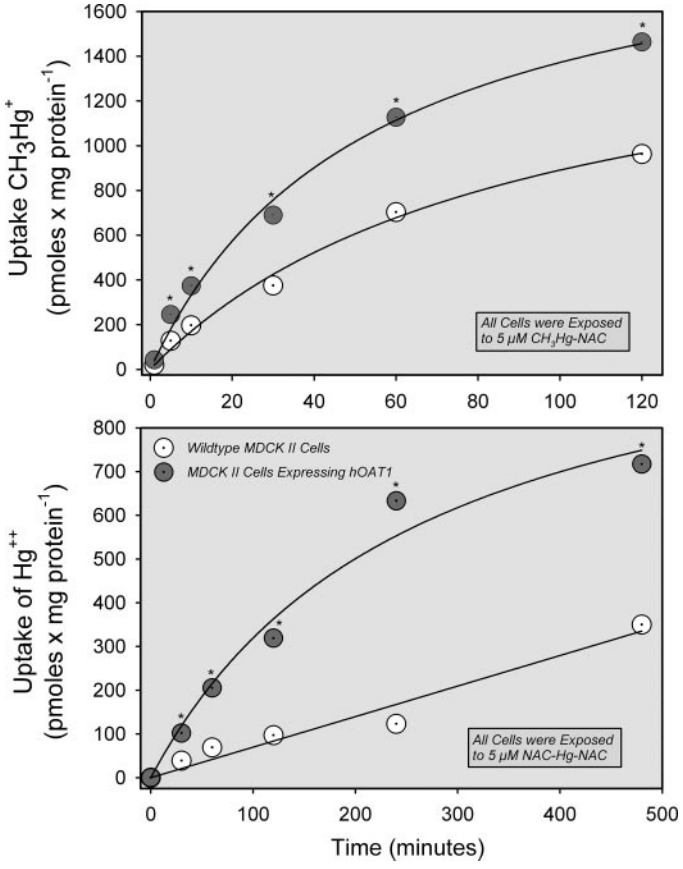

Fig. 3. Time-dependent uptake (picomoles × milligram of cellular protein^{-1}) of (A) CH_3Hg^+ or (B) Hg^{2+} in control and hOAT1-expressing MDCK II cells exposed to 5 μM CH_3Hg^+-*S*-NAC or NAC-Hg-NAC, respectively. Values are mean ± S.E. ∗, significantly different ($p < 0.05$) from the mean for the corresponding group of control MDCK cells.

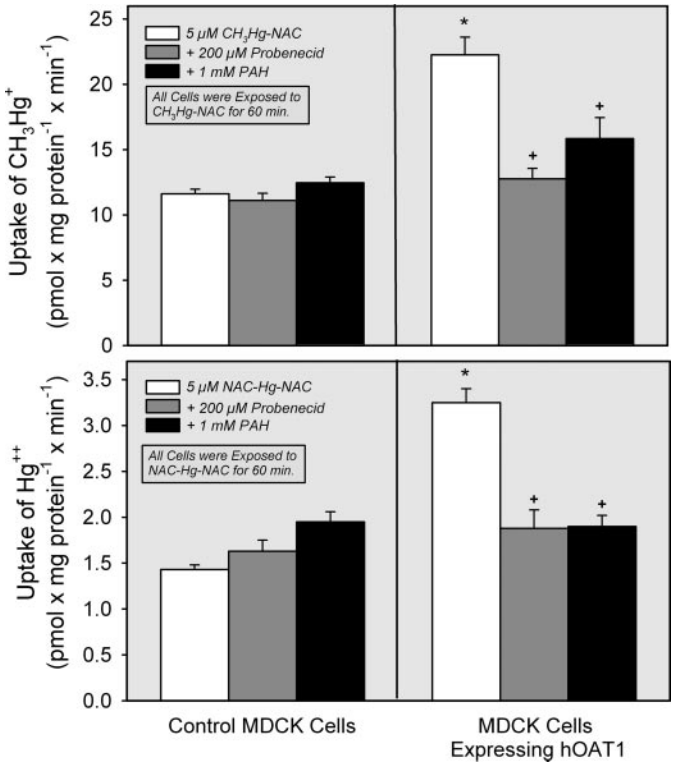

Fig. 4. Influence of 1 mM PAH or 200 μM probenecid on the uptake (picomoles × minute^{-1} × milligram of cellular protein^{-1}) of (A) CH_3Hg^+ or (B) Hg^{2+} in control and hOAT1-expressing MDCK II cells exposed to 5 μM CH_3Hg-NAC or NAC-Hg-NAC, respectively, for 60 min (at 37 °C). Values are mean ± S.E. ∗, significantly different ($p < 0.05$) from the mean for the corresponding group of wild-type control MDCK cells. +, significantly different ($p < 0.05$) from the mean for the corresponding group of hOAT1-expressing MDCK cells not exposed to either PAH or probenecid.

probenecid to the extracellular compartment containing 5 μM CH_3Hg-NAC caused the uptake of CH_3Hg^+ to be inhibited during 60 min of exposure in the hOAT1-transfected cells only (Fig. 4). By contrast, the addition of PAH or probenecid did not affect significantly the uptake of CH_3Hg^+ in the control cells when they were exposed to CH_3Hg-NAC.

Interestingly, 1 mM PAH or 200 μM probenecid also caused the uptake of Hg^{2+}, in the form of 5 μM NAC-Hg-NAC, to be inhibited in the hOAT1-transfected cells (Fig. 4). As was the case with the uptake of CH_3Hg^+, PAH or probenecid did not significantly affect the uptake of Hg^{2+} in the control cells exposed to 5 μM NAC-Hg-NAC.

Effect of Dicarboxylates on the Uptake of CH_3Hg^+ and Hg^{2+}. The uptake of CH_3Hg^+ was significantly greater in the hOAT1-transfected MDCK cells exposed to 5 μM CH_3-Hg-*S*-NAC than in the corresponding wild-type control MDCK cells (Fig. 5). The addition of 1 mM adipate or glutarate to the extracellular medium had significant effects on the transport of CH_3Hg^+ only in the hOAT1-transfected MDCK cells. In these cells, the levels of uptake of CH_3Hg^+ were reduced to levels similar to those detected in the control MDCK cells not exposed to either dicarboxylate. The levels of uptake of CH_3Hg^+ among the three groups of wild-type control cells exposed to 5 μM CH_3Hg-NAC were not significantly different from one another.

A similar pattern of transport was detected among the six groups of cells exposed to 5 μM NAC-Hg-NAC. That is, the uptake of Hg^{2+} was significantly greater in the hOAT1-transfected MDCK cells than in the corresponding wild-type control MDCK cells, and the addition of 1 mM adipate or glutarate to the extracellular medium significantly reduced the transport of Hg^{2+} only in the hOAT1-transfected MDCK cells (Fig. 5). Both dicarboxylates decreased the uptake of Hg^{2+} in the hOAT1-expressing cells to a similar extent. Furthermore, the levels of uptake of Hg^{2+} among the three groups of wild-type control cells were not significantly different from one another.

Effect of Temperature on the Uptake of CH_3Hg^+ and Hg^{2+}. Significant temperature-dependent differences in the extracellular to intracellular transport of CH_3Hg^+ or Hg^{2+} were detected among the groups of corresponding hOAT1-transfected and wild-type control cells.

At 37°C, the uptake of CH_3Hg^+ or Hg^{2+} was significantly greater in the hOAT1-transfected MDCK cells than in the corresponding wild-type control MDCK cells when the cells were exposed to 5 μM CH_3Hg-NAC or NAC-Hg-NAC, respectively, during 1 h of exposure (Fig. 6).

When the extracellular temperature was reduced to 21°C, the uptake of CH_3Hg^+ or Hg^{2+} in the hOAT1-transfected and wild-type control MDCK cells was significantly lower than that detected at 37°C (Fig. 6). In addition, there was no significant difference in the rate of uptake of either CH_3Hg^+ or Hg^{2+} between the two corresponding groups of hOAT1-expressing and wild-type control cells. The transport of CH_3Hg^+ or Hg^{2+} was for the most part abolished in both the hOAT1-expressing cells and the wild-type control cells when the temperature of the extracellular medium was reduced to 4°C.

Assessment of Toxicity and Cellular Viability. During 6 h of exposure to CH_3Hg-NAC, significant decreases in the cellular viability were detected in both the hOAT1-expressing and control MDCK cells (Fig. 7). However, significantly greater decreases in survival were detected in the hOAT1-expressing cells than in the corresponding control cells at each concentration of CH_3Hg-NAC studied (Fig. 7).

In contrast to the cells exposed to CH_3Hg-NAC, significant concentration-dependent decreases in cellular viability were detected only in the hOAT1-transfected cells when the cells

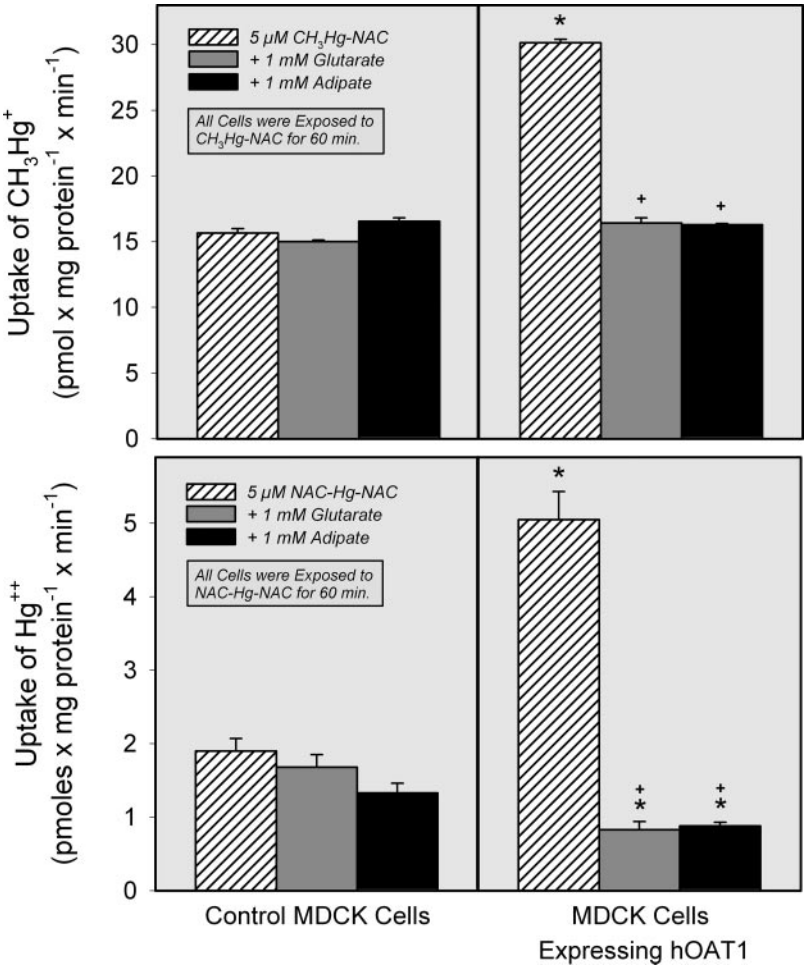

Fig. 5. Influence of 1 mM glutarate or adipate on the uptake (picomoles $\times$ minute^{-1} $\times$ milligram of cellular protein^{-1}) of (A) CH_3Hg^+ or (B) Hg^{2+} in control and hOAT1-expressing MDCK II cells exposed to 5 μM CH_3Hg-NAC or NAC-Hg-NAC, respectively, for 60 min (at 37°C). Values are mean $\pm$ S.E. *, significantly different ($p < 0.05$) from the mean for the corresponding group of wild-type control MDCK cells. +, significantly different ($p < 0.05$) from the mean for the corresponding group of hOAT1-expressing MDCK cells not exposed to either dicarboxylic acid.

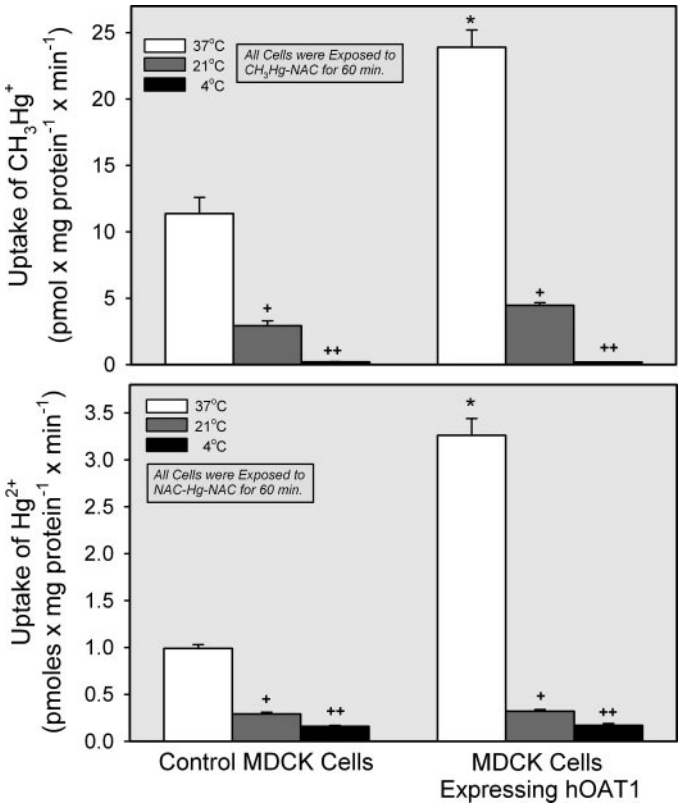

Fig. 6. Effect of temperature on the uptake (picomoles × minute^{-1} × milligram of cellular protein^{-1}) of (A) CH_3Hg^+ or (B) Hg^{2+} in control and hOAT1-expressing MDCK II cells exposed to 5 μM CH_3Hg-NAC or NAC-Hg-NAC, respectively, for 60 min. Values are mean ± S.E. *, significantly different ($p < 0.05$) from the mean for the corresponding group of wild-type control MDCK cells treated in the same manner. +, significantly different ($p < 0.05$) from the mean for the corresponding group of MDCK cells treated at 37°C. ++, significantly different ($p < 0.05$) from the mean for the corresponding group of MDCK cells treated at 37 or 21°C.

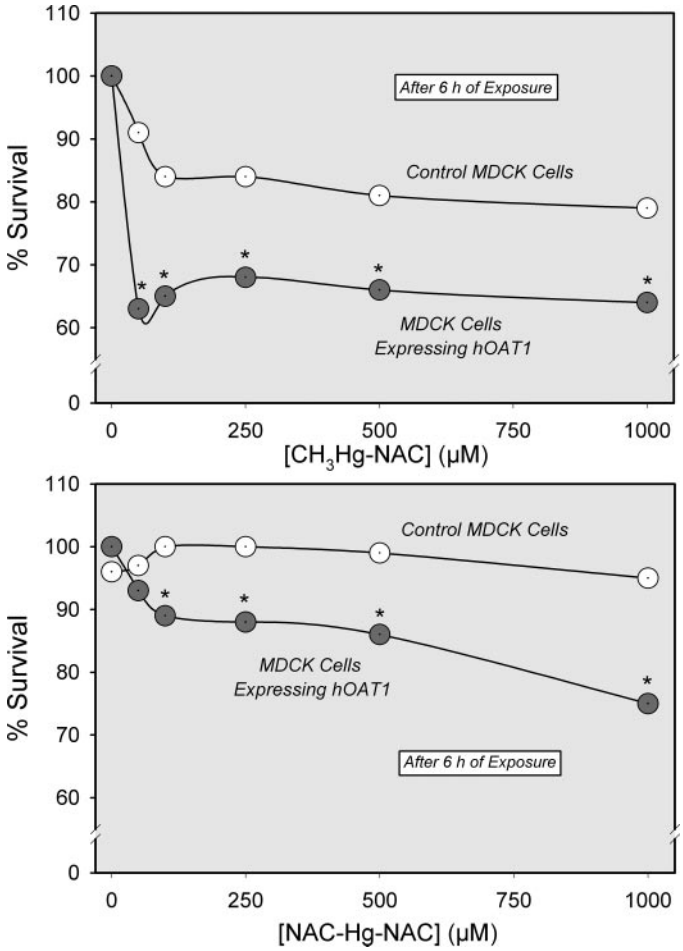

Fig. 7. The percentage of control and hOAT1-expressing MDCK II cells surviving 6 h of exposure to various concentrations of (A) CH_3Hg-NAC or (B) NAC-Hg-NAC. Values are mean ± S.E. *, significantly different ($p < 0.05$) from the mean for the corresponding group of control MDCK cells.

were exposed to NAC-Hg-NAC. The greatest differences in survival between the hOAT1-transfected and control MDCK cells were detected at concentrations of NAC-Hg-NAC greater than 100 μM.

Effect of L-Amino Acids on the Uptake of CH_3Hg^+. The uptake of CH_3Hg^+ was significantly greater in the hOAT1-transfected MDCK cells exposed to 5 μM CH_3-Hg-*S*-NAC than in the corresponding wild-type control MDCK cells (Fig. 8). The addition of 1 mM amino acids to the extracellular medium induced significant effects on the transport of CH_3Hg^+ among the groups of control and hOAT1-transfected MDCK cells. Among the control MDCK cells exposed to CH_3Hg-NAC, significant effects on the uptake of CH_3Hg^+ were detected in all groups except those exposed to 1 mM threonine, methionine, glutamate, or aspartate. Among the hOAT1-expressing cells exposed to amino acids, the levels of uptake of CH_3Hg^+ were reduced significantly in all groups except those exposed to 1 mM methionine, glutamate, or aspartate. In both the control and hOAT1-expressing cells, the addition of 1 mM L-cysteine to the extracellular compartment had the greatest effect on the uptake of CH_3Hg^+. The addition of 1 mM L-leucine had the second greatest effect on the uptake of CH_3Hg^+ in both control and hOAT1-expressing cells. Moreover, the mean level of uptake of CH_3Hg^+ in the group of hOAT1-expressing cells exposed to 5 μM CH_3Hg-NAC plus 1 mM L-leucine, L-isoleucine, L-phenylalanine, L-tyrosine, L-lysine, L-threonine, L-methionine, L-glutamate, or L-aspartate was significantly greater than that in the corresponding group of control MDCK cells treated in the same manner.

Effect of L-Leucine and/or PAH on the Uptake of CH_3Hg^+. Significant differences in the uptake of CH_3Hg^+ were again detected between the control and hOAT1-expressing MDCK cells exposed to 5 μM CH_3Hg-NAC (Fig. 9). Among the control and hOAT1-expressing MDCK cells, the uptake of CH_3Hg^+ in the group exposed to 1 mM L-leucine was significantly lower than that in the corresponding group treated with only 5 μM CH_3Hg-NAC during 30 min of exposure. Between the group of control MDCK cells and hOAT1-expressing MDCK cells exposed to 5 μM CH_3Hg-NAC and 1 mM L-leucine, the uptake of CH_3Hg^+ was greater in the group of hOAT1-expressing cells.

The addition of 1 mM PAH to the extracellular compartment did not have a significant effect on the uptake of CH_3Hg^+ in the control MDCK cells, but it did have a significant effect on the uptake of CH_3Hg^+ in the hOAT1-expressing cells. In fact, the effect of PAH was just as great as L-leucine in the hOAT1-expressing cells considering that no significant difference in the uptake of CH_3Hg^+ was detected between the group of cells treated with L-leucine and the group of cells exposed to PAH.

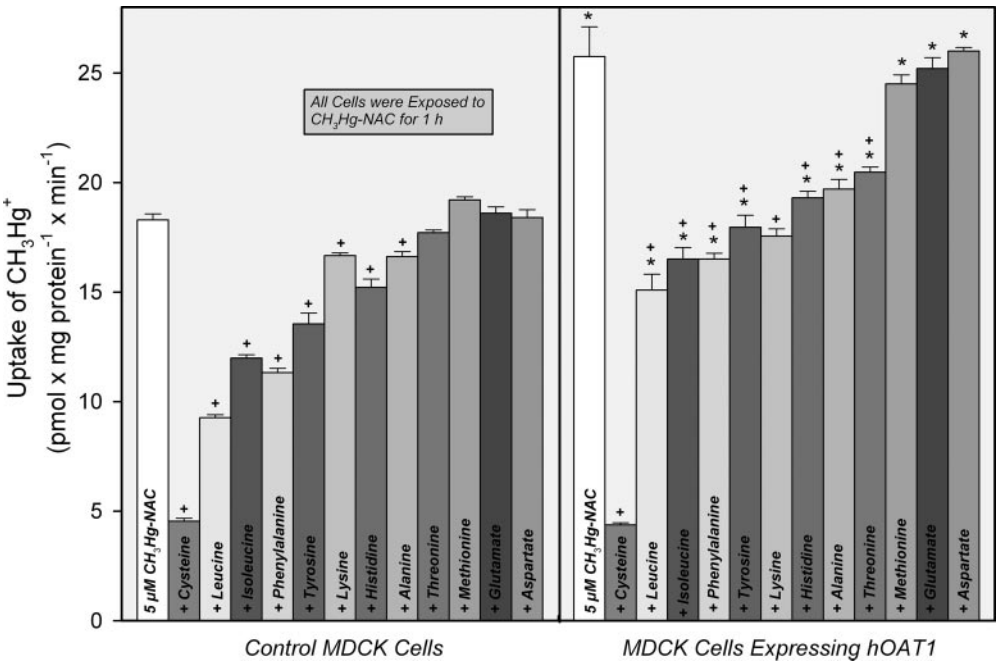

Fig. 8. Influence of extracellular amino acids (1 mM) on the uptake (picomoles $\times$ minute^{-1} $\times$ milligram of cellular protein^{-1}) of CH_3Hg^+ in control and hOAT1-expressing MDCK II cells exposed to 5 μM CH_3Hg-NAC for 60 min (at 37°C). Values are mean $\pm$ S.E. $*$, significantly different ($p < 0.05$) from the mean for the corresponding group of wild-type control MDCK cells treated in the same manner. $+$, significantly different ($p < 0.05$) from the mean for the corresponding group of control or hOAT1-expressing MDCK cells exposed to 5 μM CH_3Hg-NAC without any additional amino acids added.

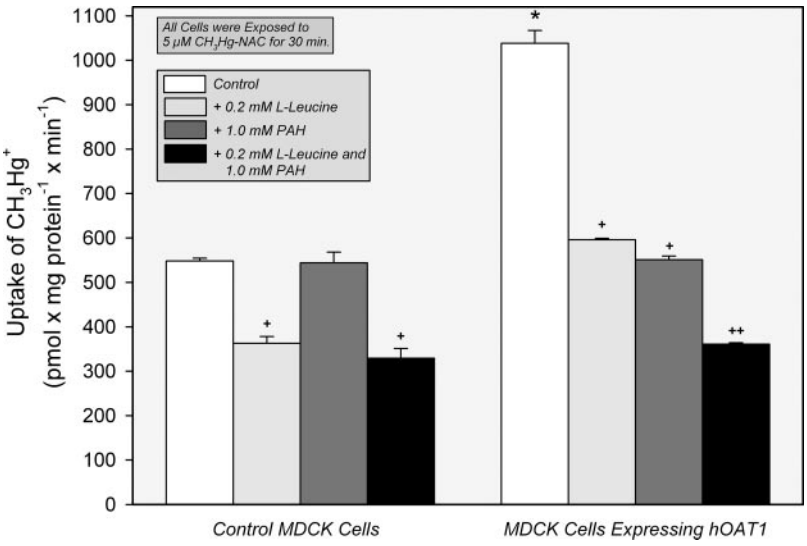

Fig. 9. Effect of adding 1 mM PAH and/or 1 mM L-leucine on the uptake (picomoles $\times$ minute^{-1} $\times$ milligram of cellular protein^{-1}) of CH_3Hg^+ in control and hOAT1-expressing MDCK II cells exposed to 5 μM CH_3Hg-NAC for 30 min (at 37°C). Values are the mean $\pm$ S.E. $*$, significantly different ($p < 0.05$) from the mean for the corresponding group of wild-type control MDCK cells treated in the same manner. $+$, significantly different ($p < 0.05$) from the mean for the corresponding group of control or hOAT1-expressing MDCK cells exposed to 5 μM CH_3Hg-NAC without any additional amino acids added. $++$, significantly different ($p < 0.05$) from the means for all corresponding groups of control or hOAT1-expressing MDCK cells.

When both PAH and L-leucine were added to the extracellular medium, an additive inhibitory effect on the uptake of CH_3Hg^+ was detected only among the groups of hOAT1-expressing MDCK cells. No significant difference in the uptake of CH_3Hg^+ was detected between the group of control MDCK cells exposed to 5 μM CH_3Hg-NAC and 1 mM L-leucine and the group of control MDCK cells exposed to 5 μM CH_3Hg-NAC, 1 mM L-leucine, and 1 mM PAH.

Discussion

Due to the continuing risk of humans being exposed to organic and inorganic forms of mercury (Zalups, 2000b), there is a growing need to better understand how the different chemical species of this metal are handled by humans and other species of mammals. To comprehend how the various forms of mercury induce their deleterious effects in a particular organism, it is paramount for one to understand how mercuric ions (in their varied forms) gain entry into the target cells they affect.

Over the past decade, a number of studies have been carried out in an attempt to characterize the disposition and transport of inorganic and organic forms of mercury in target organs (Zalups, 2000b). The findings from a number of these studies have established that the kidneys are the primary sites in the body where Hg^{2+} is taken up and accumulated and that the preponderance of this accumulation is linked to the uptake of mercuric species by proximal tubular epithelial cells (Zalups and Barfuss, 1990; Zalups, 1991a,b). More importantly, these findings indicate that at least one luminal mechanism and at least one basolateral mechanism are involved in the uptake of mercury by proximal tubular epithelial cells (Zalups, 1995, 1998a,b, 2000a; Zalups and Barfuss, 1995, 1998a,b, 2002; Zalups and Minor, 1995; Zalups and Lash, 1997; Zalups et al., 1998).

With the discovery that Hg^{2+} is taken up at the basolateral

membrane of proximal tubular epithelial cells in vivo, it was determined that much of this uptake could be inhibited by PAH (Zalups, 1995, 1998a,b, 2000a; Zalups and Barfuss, 1995, 1998b, 2002; Zalups and Minor, 1995). Because PAH is a high-affinity substrate of the dicarboxylate/organic anion exchanger OAT1 (Pritchard and Miller, 1996), it seemed logical for us to postulate that organic anion transporter(s) is involved in the basolateral uptake of mercury. However, prior to this study, very little was known about the potential role of OAT1 in the renal tubular uptake of CH_3Hg^+ (Zalups, 2000b), especially in the form of thiol *S*-conjugates.

Thus, in this study, we directly tested the hypothesis that the NAC *S*-conjugate of CH_3Hg^+ is a transportable substrate of the membrane transport protein OAT1 (specifically hOAT1). We tested this hypothesis by characterizing and comparing the transport of CH_3Hg-NAC and NAC-Hg-NAC in a line of MDCK II cells transfected stably with the cDNA encoding the hOAT1.

Prior to experimentation with mercuric *S*-conjugates of NAC, we first established that the expression and membrane insertion of a fully functional hOAT1 protein was occurring in the transfected MDCK II cells. This was accomplished by demonstrating concentration- and time-dependent transport of PAH in the hOAT1-transfected cells only and by confirming the inhibitory effects of probenecid or small dicarboxylates on the uptake of PAH (Pritchard and Miller, 1996; Zalups, 1998a, 2000b; Zalups and Barfuss, 1998b; Aslamkhan et al., 2003b; Zalups and Ahmad, 2004; Zalups et al., 2004). Afterward, we began to characterize the transport of CH_3Hg^+ and Hg^{2+}.

Our data show that, when MDCK II cells are transfected stably with hOAT1, they gain the ability to transport not only PAH but also the mercapturic acid form of CH_3Hg^+ and Hg^{2+}, i.e., CH_3Hg-NAC and NAC-Hg-NAC. The patterns of uptake of both of these mercapturic acids in the hOAT1-expressing MDCK cells were similar, although the magnitude of the inward transport of CH_3Hg-NAC was greater (on a per mole basis) than that of NAC-Hg-NAC. Analysis of saturation kinetics, time and temperature dependence, and substrate specificity in the transfected and wild-type cells demonstrates clearly that NAC *S*-conjugates of CH_3Hg^+ are indeed transportable substrates of hOAT1. We were also able to confirm the findings from a recent study in which NAC-Hg-NAC was shown to be a transportable substrate in the same cells used in the present investigation (Aslamkhan et al., 2003b). The primary rationale for including the data on NAC-Hg-NAC is for comparison and to establish reproducibility of findings in the hOAT1-transfected MDCK cells.

In proximal tubular segments in vivo, the generation (and maintenance) of a downhill, intracellular-to-extracellular gradient of α-ketoglutarate serves to facilitate the transport of organic anions into the cytosolic compartment of proximal tubular cells by a molecular exchange mechanism mediated by OAT1 (Pritchard and Miller, 1996). Other dicarboxylates, especially molecular homologs of α-ketoglutarate, such as adipate and glutarate, have been shown to compete for OAT1. The current findings show that the uptake of CH_3Hg-NAC and NAC-Hg-NAC in cells expressing hOAT1 is not only inhibited by PAH and probenecid but also by adipate or glutarate (Figs. 3 and 4). These findings, therefore, serve as additional evidence implicating NAC *S*-conjugates of CH_3Hg^+ and Hg^{2+} as transportable substrates of hOAT1.

OAT1 ostensibly has the ability to transport a broad spectrum of functionally and/or structurally similar molecules. This transport is greatly dependent on molecular structure-function relationships that exist between the transporter and substrate. Molecular homology among substrates seems to play an important role in the activity of OAT1. In support of this hypothesis are recent data implicating OAT1 in the transport of inorganic mercuric conjugates of NAC (NAC-Hg-NAC), Cys (Cys-*S*-Hg-*S*-Cys), and Hcy (Hcy-*S*-Hg-*S*-Hcy) (Pritchard and Miller, 1996; Zalups, 1998b; Zalups and Barfuss, 2002; Aslamkhan et al., 2003b; Zalups and Ahmad, 2004; Zalups et al., 2004).

Since both OAT1 and OAT3 are expressed in proximal tubular epithelial cells in vivo, one needs to consider the potential role of each of these carriers in the basolateral uptake of NAC-Hg-NAC and CH_3Hg-NAC in these cells. We recently provided evidence that NAC-Hg-NAC is a potential transportable substrate of OAT3 using *Xenopus laevis* oocytes microinjected with the cRNA for rat OAT3 (Aslamkhan et al., 2003b). After the cells began to express rat OAT3, they were shown to gain the ability to transport NAC-Hg-NAC effectively. Interestingly, when the oocytes expressing OAT3 were exposed to 5 μM NAC-Hg-NAC, the level of uptake was similar to that detected in oocytes expressing hOAT1. Thus, both OAT1 and OAT3 may transport NAC-Hg-NAC to a similar degree in proximal tubular cells in vivo. In contrast to the findings obtained from *Xenopus* oocytes expressing OAT1, Koh et al. (2002) provide data indicating that CH_3Hg-NAC may not be an effective transportable substrate in *Xenopus* oocytes expressing OAT3. These findings tend to indicate that OAT3 may not transport CH_3Hg-NAC in vivo as effectively as NAC-Hg-NAC.

Interestingly, two distinct mechanisms for the uptake of CH_3Hg-NAC were identified from the transport data obtained from the two groups of MDCK cells. The first mechanism revealed was a PAH- and dicarboxylate-sensitive component afforded exclusively to the hOAT1-transfected MDCK cells. This mechanism is also consistent with that reported recently in oocytes of *X. laevis* that were microinjected with the cRNA for rat OAT1 (Koh et al., 2002). Unexpectedly though, a significant level of uptake of CH_3Hg-NAC also occurred in the wild-type control MDCK cells. However, this uptake was not linked to a mechanism sensitive to PAH, probenecid, adipate, or glutarate, all of which are high-affinity substrates of OAT1. Clearly, a distinct mechanism not involving hOAT1 was involved in this transport. The data obtained from the study of the effects of amino acids on the transport of CH_3Hg-NAC show that this mechanism is likely mediated by one or more amino acid transporters native to MDCK cells. In both types of MDCK cells, exposure to 1 mM L-Cys, L-leucine, L-isoleucine, L-phenylalanine, or L-tyrosine had the greatest inhibitory effects on the uptake of CH_3Hg-NAC. Interestingly, the addition of L-Cys to the extracellular compartment almost abolished the transport of CH_3Hg-NAC in both cell types. Moreover, the additive effects of PAH and leucine in the hOAT1-expressing cells (Fig. 8) indicate that a combined effect of hOAT1 and one or more amino acid transporters was responsible for the overall level of uptake of CH_3Hg-NAC in these cells. Although the findings from the present study do not allow one to conclusively identify the specific amino acid transporters involved in the uptake of

CH_3Hg-NAC in the MDCK cells, potential transporters include systems L, B^0, $B^{0,+}$, and/or y^+L.

The apparent lack of participation of membrane transporters, other than hOAT1, in the uptake NAC-Hg-NAC in the MDCK cells is likely related to one or more chemical properties of this mercuric conjugate. Not only is NAC-Hg-NAC larger in molecular weight and size than CH_3Hg-NAC, but it also contains an additional negative charge that probably strongly affects the dipole moment and intramolecular dispersion and bonding forces. Each one of these factors likely affects the handling of NAC-Hg-NAC by the normal complement of transporters present in type II MDCK cells.

It is noteworthy to mention that molecular mimicry has been suggested to be involved in the uptake of Cys *S*-conjugates of CH_3Hg^+ in glial and endothelial cells (Aschner et al., 1990, 1991; Kerper et al., 1992; Clarkson, 1993; Mokrzan et al., 1995). CH_3Hg-*S*-Cys has been hypothesized to serve as a molecular mimic of the amino acid methionine at one or more neutral amino acid transporters (Clarkson, 1993). Recent data obtained from *Xenopus* oocytes expressing the neutral amino acid transporter LAT1 or LAT2 provide evidence supporting this hypothesis (Simmons-Willis et al., 2002). In the present study, molecular mimicry may also be a mechanism involved in the uptake of CH_3Hg-NAC in MDCK cells. However, the fact that methionine did not significantly affect the uptake of CH_3Hg^+ indicates that CH_3Hg-NAC does not act as a molecular mimic of methionine at the transporter(s) responsible for the uptake of CH_3Hg-NAC in wild-type MDCK II cells. LAT1 and/or LAT2 may still be involved in the uptake of CH_3Hg-NAC in the MDCK cells since L-leucine, L-isoleucine, and L-phenylalanine (which are substrates of system L transporters) were able to significantly inhibit the uptake of CH_3Hg-NAC. Considering that MDCK cells are derived from the distal nephron, the transport data obtained from the wild-type MDCK cells suggest that amino acid transporters, perhaps LAT1 and/or LAT2, may play a role in the in vivo uptake of CH_3Hg^+ in distal segments of the nephron.

It is important to note that MDCK cells have been shown to maintain polarized amino acid transport in culture (Balcarova-Stander et al., 1984). Balcarova-Stander et al. (1984) demonstrated that [^{35}S]methionine transport and protein incorporation occurred in a polarized manner in cultured MDCK cells. More specifically, they showed that uptake and protein incorporation of methionine occurred following apical or basolateral exposure to methionine, although the level of uptake and protein incorporation was much greater during basolateral exposure. These data provide support for the hypothesis that MDCK cells express a methionine (and possibly other amino acid) transporter(s), possibly system L, in a polarized manner.

Decreased survival of both wild-type control and hOAT1-expressing cells occurred following exposure to various toxic concentrations of CH_3Hg-NAC. However, the percentage of cells surviving 6 h of exposure was significantly greater in the wild-type control cells than in the hOAT1-expressing cells. These toxicological findings also support the hypothesis that at least two mechanisms were involved in the uptake of CH_3Hg-NAC in the hOAT1-expressing cells. By contrast, concentration-dependent decreases in cellular survival between the groups of MDCK cells exposed to NAC-Hg-NAC were detected only in the hOAT1-expressing cells. These data indicate that the toxic effects seen in the transfected MDCK cells were directly related to the extracellular to intracellular transport of NAC-Hg-NAC by hOAT1.

In summary, the findings from the present study represent the first line of direct molecular evidence implicating the basolateral organic anion/dicarboxylate transporter 1 and amino acid transport proteins in the cellular uptake and intoxication of NAC *S*-conjugates of CH_3Hg^+. The present findings also confirm that NAC-Hg-NAC is a transportable substrate of OAT1. Moreover, they show that NAC-Hg-NAC is not a transportable substrate of any transport proteins native to the plasma membrane of wild-type MDCK cells. Furthermore, our data indicate that OAT1 likely plays an important role in the basolateral uptake of both organic and inorganic forms of mercury along the renal proximal tubule. As a final note, this uptake may involve some form of molecular homology/mimicry.

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