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Identification of a Mechanism by Which the Methylmercury Antidotes *N*-Acetylcysteine and Dimercaptopropanesulfonate Enhance Urinary Metal Excretion: Transport by the Renal Organic Anion Transporter-1

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ABSTRACT

N-Acetylcysteine (NAC) and dimercaptopropanesulfonate (DMPS) are sulfhydryl-containing compounds that produce a dramatic acceleration of urinary methylmercury (MeHg) excretion in poisoned animals, but the molecular mechanism for this effect is unknown. NAC and DMPS are themselves excreted in urine in high concentrations. The present study tested the hypothesis that the complexes formed between MeHg and these anionic chelating agents are transported from blood into proximal tubule cells by the basolateral membrane organic anion transporters (Oat) 1 and Oat3. *Xenopus laevis* oocytes expressing rat Oat1 showed increased uptake of [^{14}C]MeHg when complexed with either NAC or DMPS but not when complexed with L-cysteine, glutathione, dimercaptosuccinate, penicillamine, or γ-glutamylcysteine. In contrast, none of these MeHg complexes were transported by Oat3-expressing oocytes. The apparent K_m values for Oat1-mediated transport were 31 ± 2 μM for MeHg-NAC and 9 ± 2 μM for MeHg-DMPS, indicating that these are relatively high-affinity substrates. Oat1-mediated uptake of [^{14}C]MeHg-NAC and [^{14}C]MeHg-DMPS was inhibited by prototypical substrates for Oat1, including *p*-aminohippurate (PAH), and was *trans*-stimulated when oocytes were preloaded with 2 mM glutarate but not glutamate. Conversely, efflux of [^{3}H]PAH from Oat1-expressing oocytes was *trans*-stimulated by glutarate, PAH, NAC, DMPS, MeHg-NAC, MeHg-DMPS, and a mercapturic acid, indicating that these are transported solutes. [^{3}H]PAH uptake was competitively inhibited by NAC (K_i of 2.0 ± 0.3 mM) and DMPS (K_i of 0.10 ± 0.02 mM), providing further evidence that these chelating agents are substrates for Oat1. These results indicate that the MeHg antidotes NAC and DMPS and their mercaptide complexes are transported by Oat1 but are comparatively poor substrates for Oat3. This is the first molecular identification of a transport mechanism by which these antidotes may enhance urinary excretion of toxic metals.

Methylmercury (MeHg) is a common environmental pollutant and a potent neurotoxicant (Clarkson, 2002). Once ingested, MeHg rapidly distributes to all tissues within the body, including its target organ, the brain (Clarkson, 1972). The only way to prevent or ameliorate toxicity once MeHg has been ingested is to accelerate its elimination from the body. Strategies for removing methylmercury include hemodialysis, exchange transfusion, and chelation therapy, with the latter being the least invasive and most common therapeutic intervention (Al-Abbasi et al., 1978; Clarkson et al., 1981; Elhassani, 1982; Lund et al., 1984). One agent that has been used successfully for chelation therapy in humans is the sulfhydryl-containing anionic compound dimercaptopropanesulfonate (DMPS). More recent studies in experimental animals indicate that a relatively simple, nontoxic amino acid derivative, *N*-acetylcysteine (NAC), may be even more effective in enhancing MeHg elimination in poisoned animals (Ballatori et al., 1998a,b). Mice that received NAC in the drinking water starting at 48 h after MeHg administration excreted from 47 to 54% of the MeHg dose in urine over the subsequent 48 h, compared with 4 to 10% in control animals (Ballatori et al., 1998b).

Although NAC and DMPS both produce a profound acceleration of urinary MeHg excretion, the mechanism for this effect is unknown. NAC and DMPS are themselves excreted in urine at high concentrations (Borgstrom et al., 1986;

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ABBREVIATIONS: MeHg, methylmercury; DMPS, dimercaptopropanesulfonic acid; NAC, *N*-acetyl-L-cysteine; Oat, organic anion transporter; PAH, *p*-aminohippuric acid.

Aposhian et al., 1995; Ballatori et al., 1998b). The present study tested the hypothesis that the anionic MeHg-NAC and MeHg-DMPS complexes are cleared from peritubular blood by kidney organic anion transporters, in particular by Oat1 and Oat3 (Lopez-Nieto et al., 1997; Sekine et al., 1997; Sweet et al., 1997; Wolff et al., 1997; Cha et al., 2001). To date, Oat1 and Oat3 are the only organic anion transporters that have been localized to the basolateral membrane of proximal tubules (Tojo et al., 1999; Sekine et al., 2000). Oat1 is localized to the S2 region of the renal proximal tubule (Tojo et al., 1999) and functions to take up a range of relatively small hydrophilic organic anions in exchange for intracellular α-ketoglutarate (Sweet and Pritchard, 1999). Uwai et al. (1998) demonstrated that hydrophilic dicarboxylates with a five-carbon backbone or longer, but not those with only three or four carbons, are able to inhibit Oat1-mediated *p*-aminohippurate (PAH) transport. In contrast, the preferred substrates for Oat3 are larger and more hydrophobic compounds such as estrone sulfate, and this transporter does not seem to function as an α-ketoglutarate exchanger (Cha et al., 2001).

The present results demonstrate that MeHg-NAC and MeHg-DMPS are high-affinity substrates for Oat1 but are comparatively poor substrates for Oat3, indicating that Oat1 may provide a route of MeHg entry into the renal tubular cells in animals treated with these therapeutic agents.

Materials and Methods

Reagents and Animals. [^{3}H]PAH (4.08 Ci/mmol) and [^{3}H]estrone sulfate (40 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA), and [^{14}C]MeHg (20.2 mCi/mmol) from American Radiolabeled (St. Louis, MO). γ-Glu-cys was a gift from the Kohjin Co. (Tokyo, Japan). Other chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO) or J.T. Baker (Philipsburg, NJ). Mature *Xenopus laevis* frogs were purchased from Nasco (Fort Atkinson, WI). Animals were maintained under a constant light cycle at a room temperature of 18°C.

Synthesis of Capped cRNA. The cDNAs for rat Oat1 and Oat3 were prepared as described previously (Sweet et al., 1997; Li et al., 1998). Capped cRNA was transcribed in vitro with T7 RNA polymerase (Ambion, Austin, TX), the cRNA was precipitated with lithium chloride, and resuspended in RNase-free water for oocyte injection. When electrophoresed on an RNA gel, these cRNAs gave single bands corresponding to the predicted sizes for Oat1 and Oat3 messages.

***X. laevis* Oocyte Preparation and Microinjection.** Isolation of *X. laevis* oocytes was performed as described by Goldin (1992) and previously employed in our laboratory (Ballatori et al., 1996; Li et al., 1998). Frogs were anesthetized by immersion for 15 min in ice-cold water containing 0.3% tricaine (Sigma-Aldrich). Oocytes were removed from the ovary and washed with OR-2 solution (82.5 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, and 5 mM HEPES-Tris, pH 7.5) and incubated at room temperature with gentle shaking for 90 min in OR-2 solution supplemented with 2 mg/ml of collagenase (type IA; Sigma-Aldrich). Oocytes were transferred to fresh collagenase solution after the first 45 min of incubation. Collagenase was removed by extensive washing in OR-2 solution at room temperature. Stage V and VI defolliculated oocytes were selected and incubated at 18°C in modified Barth's solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, and 20 mM HEPES-Tris, pH 7.5], supplemented with gentamicin (0.5 mg/ml). After 2 h of incubation, oocytes were injected with 50 nl of Oat1 or Oat3 cRNA (10–20 ng/oocyte), or sterile water for controls. Injected oocytes were cultured at 18°C with a daily change of modified Barth's medium containing gentamicin. Healthy oocytes with a clean, brown animal half and a distinct equator line were selected for experiments.

Transport Measurements in Oocytes. Uptake studies were performed 3 days after injection of cRNA. Six oocytes were incubated at 25°C for 1 h in 100 μl of modified Barth's solution in the presence of [^{3}H]PAH or of various [^{14}C]MeHg-mercaptide complexes and other substrates as described in the figure legends. Uptake was stopped by adding 2.5 ml of ice-cold modified Barth's solution, and oocytes were washed three times each with 2.5 ml of ice-cold modified Barth's solution. Two oocytes were placed in a polypropylene scintillation vial and were dissolved with 0.2 ml of 10% SDS. Radioisotope was counted in a Beckman model 6500 scintillation counter (Beckman Coulter, Fullerton, CA) after addition of 5 ml of Opti-Fluor (Packard Instruments, Downers Grove, IL).

Initial rate determinations of substrate uptake were verified from preliminary experiments demonstrating linear substrate uptake versus time for at least 1 h at the substrate concentrations tested. To preload with 2 mM glutarate or glutamate, oocytes were microinjected with 50 nl of a 22 mM stock of glutarate or glutamate, or water for control. After injection, the oocytes were incubated at room temperature for 20 to 30 min, washed three times with modified Barth's solution, and uptake of either [^{3}H]PAH, [^{14}C]MeHg-NAC, or [^{14}C]MeHg-DMPS was measured for 1 h at 25°C. To preload with [^{3}H]PAH, they were incubated with 20 μM [^{3}H]PAH for 2 h (1 μCi/ml). After this incubation, the oocytes were washed three times in modified Barth's solution, and efflux was measured over the next 60 min in modified Barth's solution containing the substrates or inhibitors described in the figure legends.

Statistical Analyses. Data were analyzed by analysis of variance (analysis of variance), and the two-tailed Student's *t* test was used for comparisons with a control. P values < 0.05 were considered statistically significant.

Results

MeHg-NAC and MeHg-DMPS Are Substrates for Oat1 but Are Poor Substrates for Oat3. Rat Oat1 or Oat3 cRNA was injected into *X. laevis* oocytes, and functional expression was assessed 3 days later by measuring uptake of [^{3}H]PAH or [^{3}H]estrone sulfate (Figs. 1A and 2A, respectively). PAH uptake was enhanced in Oat1-expressing oocytes (Fig. 1A), and estrone sulfate uptake was enhanced in Oat3-expressing oocytes (Fig. 2A). Although not shown in Fig. 2, PAH is also a substrate for Oat3 but it is transported with a low catalytic efficiency compared with estrone sulfate (Sweet et al., 2002).

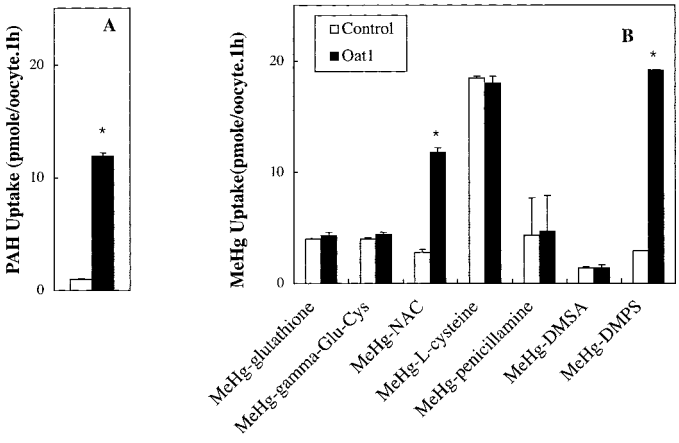

Fig. 1. Uptake of [^{3}H]PAH or of [^{14}C]MeHg mercaptide complexes in *X. laevis* oocytes expressing rat Oat1. Oocytes were injected with 20 ng of Oat1 cRNA, cultured for 3 days, and then incubated for 1 h with 20 μM [^{3}H]PAH (A) or 20 μM [^{14}C]MeHg (B) in the presence of 100 μM amino acids and thiols. DMSA, dimercaptosuccinic acid. Values are means ± S.E., $n = 3$ independent experiments, each performed in triplicate. *, $P < 0.05$; significantly different from control.

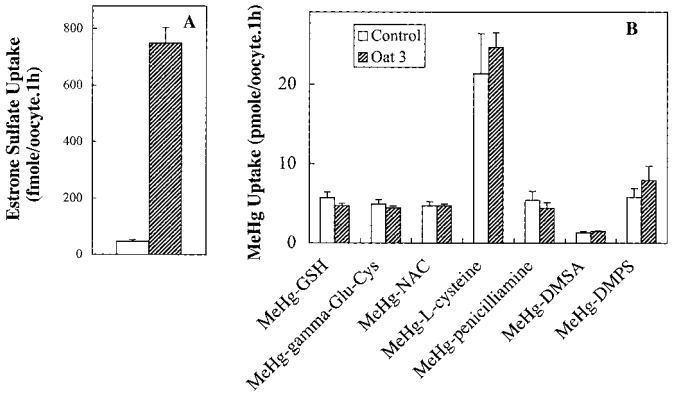

Fig. 2. Uptake of [^{3}H]estrone sulfate or of [^{14}C]MeHg mercaptide complexes in Oat3- expressing oocytes. Oocytes were injected with 20 ng of Oat3 cRNA, cultured for 3 days, and then incubated for 1 h with 50 nM [^{3}H]estrone sulfate (A) or 20 μM [^{14}C]MeHg (B) in the presence of 100 μM amino acids, organic anions, and thiols. Values are means ± S.E., n = 3 independent experiments, each performed in triplicate.

Likewise, estrone sulfate is a relatively poor substrate for Oat1 (Sweet et al., 2002).

Oat1-expressing oocytes demonstrated enhanced uptake of [^{14}C]MeHg when complexed with either NAC or DMPS, whereas [^{14}C]MeHg uptake was unaffected when complexed with glutathione (GSH), γ-Glu-Cys, L-cysteine, penicillamine, or dimercaptosuccinate (Fig. 1B). [^{14}C]MeHg uptake rates in oocytes incubated with 20 μM [^{14}C]MeHg-NAC or 20 μM [^{14}C]MeHg-DMPS were comparable with those for 20 μM PAH (Fig. 1), suggesting that these MeHg complexes are excellent substrates for Oat1. Figure 1B also demonstrates that uptake of the MeHg-L-cysteine complex was high in both control and Oat1-expressing oocytes. The observed accumulation reflects the function of endogenous oocyte amino acid transporters that accept MeHg-L-cysteine as a substrate (Simmons-Willis et al., 2002).

In contrast to Oat1, there was no significant enhancement of MeHg uptake in Oat3-expressing oocytes incubated with MeHg-NAC, MeHg-DMPS, or any of the other complexes tested (Fig. 2B).

Kinetics of MeHg-NAC and MeHg-DMPS Uptake by Oat1. Kinetic analysis of [^{14}C]MeHg-NAC uptake further suggested that it is a high-affinity substrate for Oat1, with an apparent K_m value of 31 ± 2 μM (Fig. 3A). An even higher affinity for the MeHg-DMPS complex is suggested by an apparent K_m of 9 ± 2 μM (Fig. 3B). These K_m values are comparable with those for PAH uptake on Oat1, which range from 5 to 70 μM (Sweet and Pritchard, 1999). The V_{max} values for MeHg-NAC and MeHg-DMPS were similar (22 ± 4 and 20 ± 3 pmol/oocyte/h, respectively; Fig. 3).

Substrate and Inhibitor Specificity of Oat1. The substrate and inhibitor specificity of Oat1was assessed by measuring uptake of [^{3}H]PAH, [^{14}C]MeHg-NAC, and [^{14}C]MeHg-DMPS in the presence of the compounds shown in Fig. 4. Oat1-mediated transport of all three substrates was markedly reduced by PAH, probenecid, *S*-dinitrophenyl-NAC, and butyl-NAC but was unaffected by the neutral amino acids L-alanine and L-methionine. *N*-Acetyl-L-methionine and ethyl-NAC also reduced uptake of these three substrates. In addition, DMPS and NAC were *cis*-inhibitors of PAH uptake (Fig. 4), suggesting that they may be potential substrates.

To test the hypothesis that DMPS and NAC are substrates for Oat1, the kinetics of their inhibition was examined (Fig. 5). The results support this hypothesis by demonstrating that DMPS and NAC are competitive inhibitors of PAH uptake,

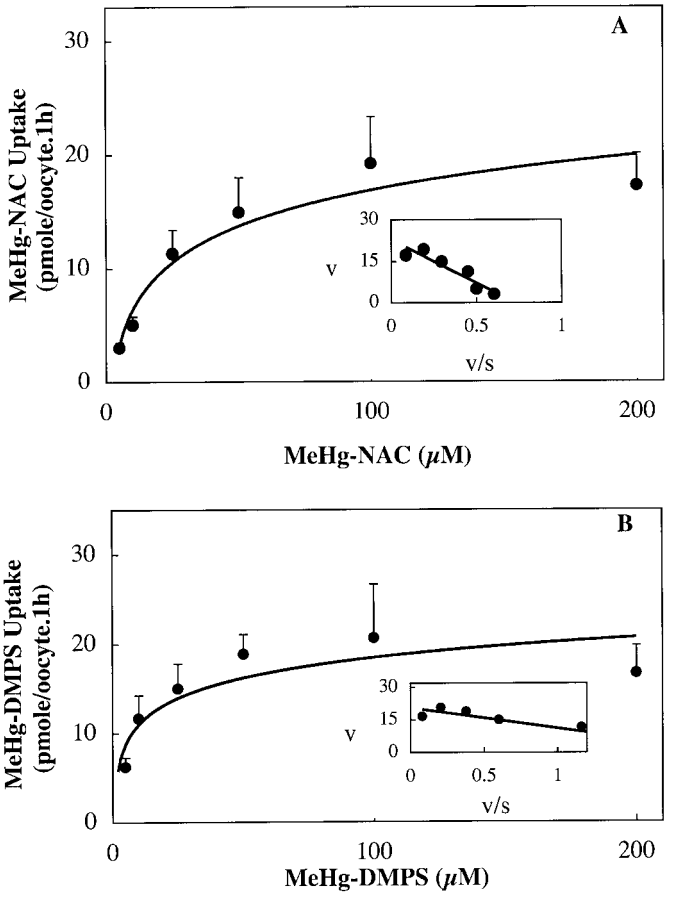

Fig. 3. Concentration dependence of [^{14}C]MeHg-NAC and [^{14}C]MeHg-DMPS uptake rates in Oat1-expressing oocytes. Oocytes were incubated in MeHg-mercaptide concentrations of 5, 10, 25, 50, 100, and 200 μM with a thiol to MeHg ratio of 3:1. Insets illustrate Eadie-Hofstee plots of substrate uptake measured at 1 h. Data points represent values for Oat1-expressing oocytes (20 ng cRNA) minus water-injected oocytes. Values are means ± S.E., n = 3 separate experiments, each performed in triplicate.

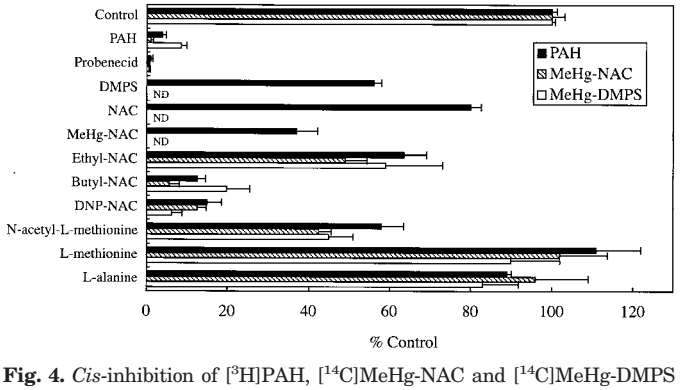

Fig. 4. *Cis*-inhibition of [^{3}H]PAH, [^{14}C]MeHg-NAC and [^{14}C]MeHg-DMPS uptake into Oat1-expressing oocytes by organic anions and mercapturic acids. Oocytes were exposed to 20 μM [^{3}H]PAH, 10 μM[^{14}C] MeHg-NAC, and 10 μM [^{14}C]MeHg-DMPS in the absence (control) or presence of various compounds for 1 h. All inhibitors were present at a concentration of 1 mM, except for *S*-dinitrophenyl-NAC which was at 0.1 mM. Uptake values in water-injected oocytes were subtracted from those of Oat1-expressing oocytes. Values are means ± S.E. of four separate experiments, each performed in triplicate. All values are significantly different from control, $P < 0.05$ by Student's t test, except those for L-methionine and L-alanine.

with K_i values of 0.10 ± 0.02 and 2.0 ± 0.3 mM, respectively (Fig. 5).

***trans*-Stimulation of MeHg-NAC and MeHg-DMPS Transport on Oat1.** Oat1 has been shown to function as a dicarboxylate-coupled anion exchanger (Sweet and Pritchard, 1999). To confirm that Oat1 mediates uptake of MeHg-complexes by exchange with specific intracellular dicarboxylates, the effect of an imposed glutarate gradient on MeHg transport was assessed. Oat1-mediated uptake of [^{3}H]PAH, [^{14}C]MeHg-NAC, and [^{14}C]MeHg-DMPS was measured in oocytes that were microinjected with glutarate or glutamate to obtain intracellular concentrations of approximately 2 mM, or water for control. Figure 6 shows that uptake of all three substrates by Oat-1 expressing oocytes was increased when intracellular glutarate was added, but not when glutamate was added. The observed dicarboxylate-induced *trans*-stimulation of substrate uptake indicates that Oat1 mediates uptake of MeHg-NAC and MeHg-DMPS by exchange with intracellular dicarboxylates.

Oat1-mediated anion exchange of MeHg-NAC and MeHg-DMPS was further verified by assessing *trans*-stimulation of [^{3}H]PAH efflux (Fig. 7). Oocytes were preloaded with [^{3}H]PAH and efflux was measured in media containing the substrates shown in Fig. 7. Efflux of [^{3}H]PAH was enhanced by MeHg-NAC and MeHg-DMPS, as well as by DMPS, NAC, PAH, glutarate, and butyl-NAC, whereas the neutral amino acid L-alanine had no effect. The *trans*-stimulation of PAH efflux provides additional evidence for Oat1-mediated exchange of MeHg-NAC and MeHg-DMPS and also indicates that NAC and DMPS are transported substrates for Oat1.

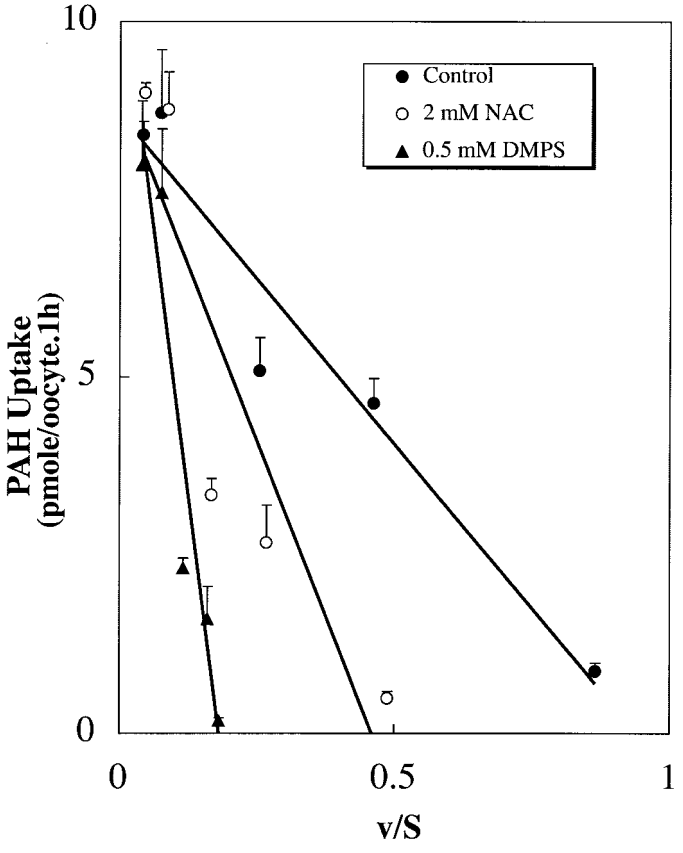

Fig. 5. NAC and DMPS competitively inhibit Oat1-mediated [^{3}H]PAH uptake in oocytes. Uptake of increasing concentrations of [^{3}H]PAH was measured in the absence (control) or in the presence of 2 mM NAC or 0.5 mM DMPS. Uptake values in water-injected oocytes were subtracted from those of Oat1-expressing oocytes. Data represent the mean ± S.E. of three separate experiments.

Discussion

DMPS and NAC are organic anions that produce a dramatic acceleration of MeHg excretion in urine and are themselves excreted in urine at high concentrations. To account for this effect, the present study tested whether DMPS, NAC, and their corresponding MeHg complexes are substrates for Oat1 and Oat3, which are localized to the basolateral membrane of rat proximal tubule cells (Kojima et al., 2002). Our

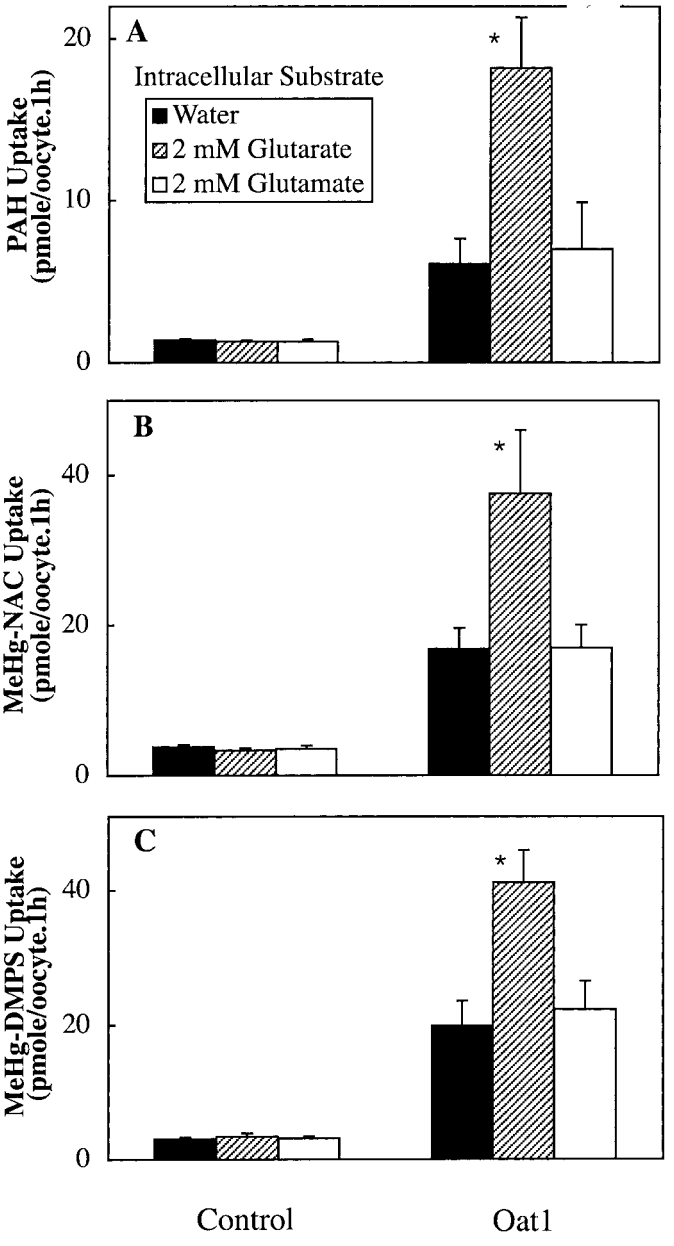

Fig. 6. *trans*-Stimulation of 20 μM [^{3}H]PAH, 20 μM [^{14}C]MeHg-NAC, and 20 μM [^{14}C]MeHg-DMPS uptake in Oat1-expressing oocytes by intracellular glutarate. Uptake was measured for 1 h in both control and Oat1-expressing oocytes that had been preloaded with 2 mM glutamate, 2 mM glutamate, or water for control. Values are means ± S.E., $n = 3$ separate experiments, each performed in triplicate. *, $P < 0.05$; significantly different from control.

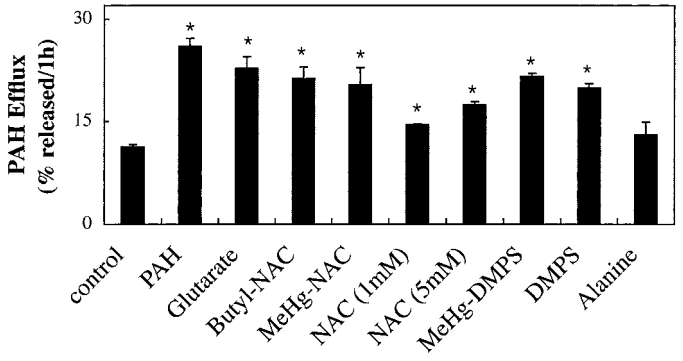

Fig. 7. *trans*-Stimulation of [^{3}H]PAH efflux in Oat1-expressing oocytes by extracellular substrates. Efflux was measured for 1 h in the presence of the compounds shown. All compounds were present at a concentration of 1 mM, except for NAC, which was tested at both 1 and 5 mM. Values are means ± S.E., $n = 6$ independent experiments, each performed in quadruplicate. *, $P < 0.05$; significantly different from control.

results demonstrate that MeHg-NAC and MeHg-DMPS are high-affinity substrates for Oat1 but are comparatively poor substrates for Oat3, and indicate that NAC and DMPS are also substrates for Oat1. These results identify a molecular mechanism by which these therapeutic agents enhance whole-body elimination of this toxic metal.

The present results also provide insight into the substrate specificity for Oat1. Specifically, these findings support the conclusion that carbon chain length is an important determinant of Oat1-mediated transport selectivity (Uwai et al., 1998; Pombrio et al., 2001). Uwai et al. (1998) demonstrated that dicarboxylates with a 5-carbon backbone or longer, but not those with only three or four carbons, are able to inhibit Oat1-mediated PAH transport. The present results indicate that MeHg-NAC, MeHg-DMPS, NAC, and DMPS, all of which have a molecular backbone containing five or more atoms (Fig. 8), are substrates for Oat1. In contrast, MeHg complexes with dimercaptosuccinic acid, penicillamine, and acetylpenicillamine, which have a molecular backbone containing only four atoms (Fig. 8), do not seem to interact with Oat1.

Although the present study did not directly measure Oat1-mediated transport of NAC and DMPS, several lines of evidence indicate that these chelating agents are themselves substrates for Oat1. As noted above, these molecules possess the key structural features, namely a net negative charge and a five-atom backbone (Fig. 8). Moreover, NAC and DMPS were able to *cis*-inhibit (Fig. 4) and *trans*-stimulate PAH transport (Fig. 6), and both were competitive inhibitors of PAH uptake (Fig. 5). It is interesting to note that the K_i values for inhibition of PAH transport by NAC and DMPS were 2 and 0.1 mM, respectively, suggesting that they have a low affinity for Oat1 compared with PAH (K_m, 5 to 70 μM; Sweet et al., 2002), MeHg-NAC (K_m, 31 ± 2 μM), and MeHg-DMPS (K_m, 9 ± 2 μM). Thus, the addition of MeHg to NAC or DMPS seems to enhance affinity for the transporter, an effect that may be attributed to the increased hydrophobic character and molecular size of the resulting MeHg complexes (Fig. 8). This higher apparent affinity of the mercaptide complexes suggests that they are transported more efficiently than the parent chelating agents, which in turn would promote more MeHg complexation and excretion.

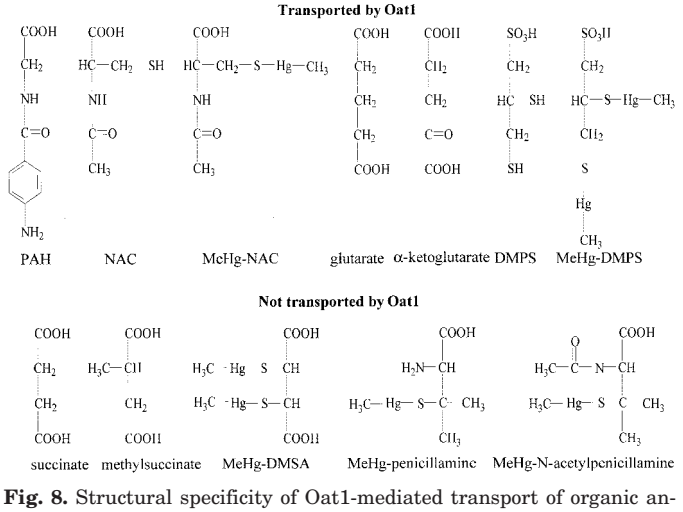

Fig. 8. Structural specificity of Oat1-mediated transport of organic anions. Substrates for Oat1 seem to have a molecular backbone containing at least five atoms, whereas compounds that have a four-atom backbone do not seem to be transported.

Oat1 is known to function as an α-ketoglutarate–coupled anion exchanger at the basolateral membrane of the proximal tubule. The α-ketoglutarate gradient across the basolateral membrane is large and provides a powerful driving force for organic anion uptake via Oat1. Indeed, the magnitude of this driving force is sufficiently large to account for efficient organic anion secretion, often mediating substrate clearance in a single pass through the kidney. These observations suggest that Oat1 mediates the initial active step in the process of organic anion secretion across the proximal tubule. The present results demonstrate that Oat1 is an anion exchanger coupling uptake of MeHg-NAC and MeHg-DMPS to gradients of glutarate, PAH, and mercapturic acids (Figs. 6 and 7).

Upon their uptake into proximal tubule cells, urinary secretion of organic anions requires efflux across the brush border membrane. However, the transporter(s) mediating export across the brush border membrane are presently unknown. Candidate transporters include the multidrug resistance-associated protein-2 (Mrp2), which has been localized to the brush border membrane (Schaub et al., 1997). In hepatocytes, Mrp2 functions to transport glutathione, glutathione *S*-conjugates, glutathione mercaptides, and other organic anions across the canalicular membrane into bile (Ballatori, 1994, 2002). Alternatively, other members of the Oat or Oatp families of transporters, yet to be localized to the brush border membrane, may mediate efflux of MeHg complexes.

The molecular identification of a mechanism mediating MeHg transport across cell membranes advances our understanding of MeHg toxicity and possible therapy. Thus, organic anion transporters may have an important role in facilitating urinary MeHg excretion, and may contribute to individual susceptibility to MeHg toxicity. Additional studies are needed to test these possibilities.

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References

Al-Abbasi AH, Kostyniak PJ, and Clarkson TW (1978) An extracorporeal complexing hemodialysis system for the treatment of methylmercury poisoning. III. Clinical applications. *J Pharmacol Exp Ther* **207:**249–254.
Aposhian HV, Maiorino RM, Gonzalez-Ramirez D, Zuniga-Charles M, Xu Z, Hurlbut

KM, Junco-Munoz P, Dart RC, and Aposhian MM (1995) Mobilization of heavy metals by newer, therapeutically useful chelating agents. *Toxicology* **97:**23–38.
Ballatori N (1994) Glutathione mercaptides as transport forms of metals. *Adv Pharmacol* **27:**271–298.
Ballatori N (2002) Transport of toxic metals by molecular mimicry. *Environ Health Persp*, in press.
Ballatori N, Lieberman MW, and Wang W (1998a) Accelerated methylmercury elimination in γ-glutamyl transpeptidase-deficient mice. *Am J Pathol* **152:**1049–1055.
Ballatori N, Lieberman MW, and Wang W (1998b) *N*-Acetylcysteine as an antidote in methylmercury poisoning. *Environ Health Persp* **106:**267–271.
Ballatori N, Wang W, Li L, and Truong AT (1996) An endogenous ATP-sensitive glutathione *S*-conjugate efflux mechanism in *Xenopus laevis* oocytes. *Am J Physiol* **270:**R1156–R1162.
Borgstrom L, Kagedal B, and Paulsen O (1986) Pharmacokinetics of *N*-acetylcysteine in man. *Eur J Clin Pharmacol* **31:**217–222.
Cha SH, Sekine T, Fukushima JI, Kanai Y, Kobayashi Y, Goya T, and Endou H (2001) Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol Pharmacol* **59:**1277–1286.
Clarkson TW (1972) The pharmacology of mercury compounds. *Annu Rev Pharmacol* **12:**375–406.
Clarkson TW (2002) The three modern faces of mercury. *Environ Health Perspect* **110 (Suppl 1):**11–23.
Clarkson TW, Magos L, Cox C, Greenwood MR, Amin-Zaki L, Majeed MA, and Al-Damluji SF (1981) Tests of efficacy of antidotes for removal of methylmercury in human poisoning during the Iraq outbreak. *J Pharmacol Exp Ther* **218:**74–83.
Elhassani SB (1982) The many faces of methylmercury poisoning. *J Toxicol Clin Toxicol* **19:**875–906.
Goldin AL (1992) Maintenance of *Xenopus laevis* and oocyte injection. *Methods Enzymol* **207:**266–279.
Kojima R, Sekine T, Kawachi M, Cha SH, Suzuki Y, and Endou H (2002) Immunolocalization of multispecific organic anion transporters, OAT1, OAT2 and OAT3, in rat kidney. *J Am Soc Nephrol* **13:**848–857.
Li L, Lee TK, Meier PJ, and Ballatori N (1998) Identification of glutathione as a driving force and leukotriene C_4 as a substrate for Oapt1, the hepatic sinusoidal organic solute transporter. *J Biol Chem* **273:**16184–16191.
Lopez-Nieto CE, You G, Bush KT, Barros EJG, Beier DR, and Nigam SK (1997) Molecular cloning and characterization of NKT, a gene product related to the organic cation transporter family that is almost exclusively expressed in the kidney. *J Biol Chem* **272:**6471–6478.
Lund ME, Clarkson TW, and Berlin M (1984) Treatment of acute methylmercury ingestion by hemodialysis with *N*-acetylcysteine (Mucomyst^R) infusion and 2,3-dimercaptopropane sulfonate. *Clin Toxicol* **22:**31–49.
Pombrio JM, Giangreco A, Li L, Wempe MF, Anders MW, Sweet DH, Pritchard JB, and Ballatori N (2001) Mercapturic acids (*N*-acetylcysteine *S*-conjugates) as endogenous substrates for the renal organic anion transporter-1, Oat1. *Mol Pharmacol* **60:**1091–1099.
Schaub TP, Kartenbeck J, Konig J, Vogel O, Witzgall R, Kriz W, and Keppler D (1997) Expression of the conjugate export pump encoded by the mrp2 gene in the apical membrane of kidney proximal tubules. *J Am Soc Nephrol* **8:**1213–1221.
Sekine T, Cha SH, and Endou H (2000) The multispecific organic anion transporter (OAT) family. *Pfleug Arch Eur J Physiol* **440:**337–350.
Sekine T, Watanabe N, Hosoyamada M, Kanai Y, and Endou H (1997) Expression cloning and characterization of a novel multispecific organic anion transporter. *J Biol Chem* **272:**18526–18529.
Simmons-Willis TA, Koh AS, Clarkson TW, and Ballatori N (2002) Transport of a neurotoxicant by molecular mimicry: the methylmercury-L-cysteine complex is a substrate for human LAT1 and LAT2 amino acid carriers. *Biochem J*, in press.
Sweet DH, Miller DS, Pritchard JB, Fujiwara Y, Beier DR, and Nigam SK (2002) Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 [Slc22a8]) knockout mice. *J Biol Chem*, in press.
Sweet DH and Pritchard JB (1999) The molecular biology of renal organic anion and organic cation transporters. *Cell Biochem Biophys* **31:**89–118.
Sweet DH, Wolff NA, and Pritchard JB (1997) Expression cloning and characterization of ROAT1. The basolateral organic anion transporter in rat kidney. *J Biol Chem* **272:**30088–30095.
Tojo A, Sekine T, Nakajima N, Hosoyamada M, Kanai Y, Kimura K, and Endou H (1999) Immunohistochemical localization of multispecific renal organic anion transporter 1 in rat kidney. *J Am Soc Nephrol* **10:**464–471.
Uwai Y, Okuda M, Takami K, Hashimoto Y, and Inui K (1998) Functional characterization of the rat multispecific organic anion transporter OAT1 mediating basolateral uptake of anionic drugs in the kidney. *FEBS Lett* **438:**321–324.
Wolff NA, Werner A, Burkhardt S, and Burckhardt G (1997) Expression cloning and characterization of a renal organic anion transporter from winter flounder. *FEBS Lett* **417:**287–291.

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