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Mouse Monocytes (RAW CELLS) and the Handling of Cysteine and Homocysteine S-Conjugates of Inorganic Mercury and Methylmercury

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Although there is evidence indicating that mononuclear phagocytes can take up mercury by some forms of endocytosis, very little is known about the potential for the uptake of mercuric species by carrier-mediated processes. Thus, we hypothesized that monocytes also possess mechanisms allowing these cells to take up inorganic mercury (Hg^{2+}) and/or methylmercury (CH_3Hg^+) as cysteine (Cys) and/or homocysteine (Hcy) S-conjugates by certain membrane transport proteins. The specific thiol S-conjugates were chosen for study because our laboratory and those of some other investigators have demonstrated that these species of mercury are indeed transportable substrates for several membrane transport proteins in certain types of epithelial cells. We chose to use RAW 264.7 cells for our experiments. These cells represent an adherent line of mouse monocytes. Kinetic analyses for the uptake of Cys-Hg-Cys, CH_3Hg-Cys, Hcy-Hg-Hcy, and CH_3Hg-Hcy revealed that uptake occurred by a saturable, concentration-dependent mechanism, displaying Michaelis–Menten properties. Interestingly, in the cells exposed to the Cys or Hcy S-conjugate of Hg^{2+}, significantly more Hg^{2+} was taken up in the presence of 140 m*M* sodium chloride (NaCl) than in the presence of 140 mM *N*-methyl-D-glucamine (NMDG), indicating that Na-dependent processes play more of a role in the uptake of these species of Hg^{2+} than sodium-independent ones. With respect to the uptake of CH_3Hg^+, rates of uptake of the Cys and Hcy S-conjugates of CH_3Hg^+ were similar under both Na-dependent and Na-independent conditions, although the levels of uptake of these mercuric species far exceeded the levels of uptake of the corresponding S-conjugate of Hg^{2+}. Uptake of Hg^{2+} and CH_3Hg^+, as the Cys or Hcy S-conjugates, was also time-dependent. We also showed that when the temperature in the bathing medium was reduced to 4°C, uptake of the Cys S-conjugates Hg^{2+} or CH_3Hg^+ was for the most part reduced to negligible levels in the RAW cells; indicating that the preponderance of uptake at 37°C was not due primarily to simple diffusion and/or non-specific binding. Overall, the present findings strongly suggest that the uptake of the Cys and Hcy S-conjugates of Hg^{2+} and/or CH_3Hg^+ occurs in monocytes by one or more mechanisms involving carrier proteins.

Monocytes represent an important population of mononuclear leukocytes in blood that play a vital role in cellular immunity, especially in innate immunity. After being activated by various conditions and molecules, including several cytokines, monocytes migrate from systemic circulation into extravascular tissues. During activation, monocytes undergo a transformation into active phagocytic macrophages, which play an important role in the immune response by engulfing and digesting foreign microorganisms and biochemicals, dead or worn out cells, and cellular debris. Monocytes/macrophages also play an important role in modulating the activity of the immune system by presenting foreign antigens to lymphocytes. Disorders affecting phagocytotic activity of macrophages can result in the failure of these cells to generate oxygen radicals (O_2^-), which is a crucial component in immune responses.

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In recent studies, activation of human monocytes, especially the production of oxygen free radicals, has been shown to be affected greatly by the group IIB metals mercury, cadmium, and zinc (Leibbrandt & Koropatnick, 1994). Both in vivo and in vitro exposure to these metals has been shown to decrease the ability of monocytes to be activated by bacterial lipopolysaccharide or phorbol ester and undergo the respiratory burst, which is characterized by increased production of superoxide radicals. Interestingly, induction of metallothionein expression, which appears to be linked to the activation of human monocytes, has also been shown to be diminished as a result of exposure to these metals (Leibbrandt & Koropatnick, 1994; Leibbrandt et al., 1994; Koropatnick & Zalups, 1997).

In another study, exposure to very low levels of metallic mercury was suggested to promote subtle impairment in circulating monocytes without apparent clinical symptoms (Vimercati et al., 2001). Although mercuric ions are believed to have the capacity to induce apoptosis in some cells (Insug et al., 1997), it has been suggested that exposure to mercuric species, at extracellular concentrations of 1 to 5 μ*M*, may result in damage to DNA, without it leading apoptosis (Ben-Ozer et al., 2000).

Many of the cellular effects of mercuric species that occur in mononuclear leukocytes are premised mainly on the ability of mercuric ions gaining entry into the cytosolic compartment of these cells. Unfortunately, very little is known about the mechanisms by which mercuric ions gain entry into monocytes and macrophages. There are some data indicating that phagocytic cells can take up mercuric species by endocytosis (Cunha et al., 2004; Cherdwongcharoensuk et al., 2002). However, very little else is known about the mechanisms by which monocyte/macrophages can take up mercuric species. This issue is very important, considering the potential impact of mercury on the immune system and the increasing levels of environmental contamination with mercuric species.

Carrier-mediated uptake of both inorganic and organic mercuric species has been implicated in various types of epithelial cells. Various sets of findings from renal tubular epithelial cells and endothelial cells indicate that cysteine (Cys) and homocysteine (Hcy) S-conjugates of inorganic mercury (Hg^{2+}) or methylmercury (CH_3Hg^+) can serve as transportable substrates of certain amino acid transporters, such as system b^{0+} (Bridges et al., 2004; Bridges & Zalups, 2004) and system L (Simmons-Willis et al., 2002; Mokrzan et al., 1995), and organic anion transporters (OAT1 and OAT3; Aslamkhan et al., 2003, Zalups & Ahmad 2004;, Zalups et al., 2004, Zalups & Ahmad, 2005). Based on these findings, it is possible that some of the same and/or different membrane transporters can participate in the uptake of inorganic and/or organic forms of mercury in monocytes and/or macrophages.

In the present study, therefore, we designed experiments to determine if certain thiol S-conjugates of Hg^{2+} and/or CH_3Hg^+ can be taken up by one or more carrier-mediated processes in monocytes. More specifically, we tested the hypothesis that cysteine (Cys) S-conjugates and homocystine (Hcy) S-conjugates of Hg^{2+} and/or CH_3Hg^+ can be taken up by monocytes by carrier-mediated mechanisms.

MATERIALS AND METHODS

Tissue Culture

RAW 264.7 cells, an adherent mouse monocyte/macrophage cell line, (ATCC, Manassas, VA) were maintained in Dulbecco's modified Eagle's medium (DMEM, ATCC) supplemented with 10% fetal bovine serum (Atlanta Biological, Atlanta, GA) and 1% penicillin streptomycin (Invitrogen, Carlsbad, CA). The cells were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Subcultures were prepared by scraping the cells and adding 1/10th of the culture to a new flask every 3–4 d.

Transport Assays

Prior to experimentation, cells were seeded into 24-well plates (Costar, NY) at a density of 0.5×10^6 cells/well and were cultured for 3 d with the media being changed daily. At the beginning of each experiment, the culture media was aspirated from the plate and the cells were washed with warm uptake buffer (140 m*M* NaCl, 25 m*M* Hepes, 5.4 m*M* KCl, 1.8 m*M* $CaCl_2$, 0.8 m*M* $MgSO_4$, 5 m*M* glucose, pH 7.5). In selective experiments, the NaCl in the buffer was replaced by 140 m*M* *N*-methyl-D-glucamine (NMDG) in order to generate Na-free conditions. Uptake of each mercuric species was initiated by adding of 250 μl of the desired buffer containing radiolabeled form of the substrate being studied.

Uptake of Hg^{2+} and CH_3Hg^+, as conjugates of Cys (Cys-Hg-Cys and CH_3Hg-Cys, respectively) and Hcy (Hcy-Hg-Hcy and CH_3Hg-Hcy, respectively) by the cells was used to evaluate transport processes under various conditions. Cys-Hg-Cys was prepared by mixing 5 μ*M* mercuric chloride ($HgCl_2$), containing the radioactive isotope of Hg^{2+}, ^{203}Hg, and 12.5 μ*M* unlabeled Cys at room temperature. Likewise, CH_3Hg-Cys was prepared by mixing 5 μ*M* CH_3Hg, as [^{14}C]-CH_3Hg, with 6 μ*M* cysteine. The same ratios were used to prepare the conjugates for homocysteine. These ratios ensure that there is adequate Cys or Hcy to which Hg or CH_3Hg can bind without adding excess amino acid to the mixture.

Cells were incubated at 37°C unless stated otherwise. Uptake of each substrate by the RAW cells was stopped by aspirating the respective radiolabeled compound and then quickly washing the cells twice with ice-cold uptake buffer containing 1 m*M* *meso*-2,3-dimercaptosuccinic acid (DMSA, Sigma, St. Louis, MO), a well-known chelator of mercuric ions (Zalups, 1993). Subsequently, the cells were solubilized in 0.5 ml 1% sodium dodecyl sulfate (SDS) in 0.2 N NaOH. Samples of the solubilized cells were placed in scintillation vials containing 5 ml Optifluor scintillation liquid (Perkin Elmer, Boston) and were counted in a Beckman Coulter LS6500 scintillation

counter (Beckman Coulter Inc. Fullerton, CA) for 5 min. The content of mercuric ions in each sample was determined by standard methods used for liquid scintillation spectrometry.

Rates of transport for kinetic analyses were factored by both time in min and cellular content of protein, which was assessed by the Bradford protein assay (Bradford, 1976).

Assessment of Saturation Kinetics

Saturation kinetics for the transport processes was evaluated by incubating cells with Cys-Hg-Cys or CH_3Hg-Cys, for 30 or 15 min, respectively, in the presence of several concentrations of unlabeled Cys-Hg-Cys (5, 10, 25, 50, 75, 100 μ*M*) or CH_3Hg-Cys (5, 50, 100, 250, 500, 1000 μ*M*). The saturation kinetics of Hcy-Hg-Hcy or CH_3Hg-Hcy were measured as described earlier for the homocysteine conjugates. Concentration-response data for the uptake of the conjugates were fitted to the Michaelis–Menten equation.

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

Here, V represents velocity, V_{max} is the maximal velocity for the transport of the substrate, [S] is the concentration of the substrate, and K_m is the Michaelis–Menten constant.

Determination of Time Dependency for the Uptake of Mercuric Conjugates

Time-course experiments were performed by incubating the cells with 5 μ*M* Cys-Hg-Cys, CH_3Hg-Cys, Hcy-Hg-Hcy, or CH_3Hg-Hcy for time periods ranging from 5 to 120 min. The 5-μ*M* concentration was chosen for study to ensure near 100% viability through the course of the experiment.

Evaluation of the Effects of Temperature on Transport

In order to determine the effect of temperature on the transport process, saturation kinetics experiments with cysteine were also performed at 4°C and 37°C simultaneously. Cells were exposed to 5 μ*M* Cys-Hg-Cys or CH_3Hg-Cys for 30 or 15 min in the presence of unlabeled Cys-Hg-Cys or CH_3Hg-Cys, respectively. We chose to only study the effects of temperature in RAW cells exposed to the Cys S-conjugates because we have observed similar effects of temperature in a number of other cell lines with the Cys S-conjugate or the Hcy S-conjugate of Hg^{2+} or CH_3Hg^+.

Evaluation of Cellular Viability

Cellular viability assays using trypan blue were conducted to determine whether Hg^{2+} and CH_3Hg^+ affected the mortality of the cells over the time-course and concentrations of substrate studied. In brief, a 25-μ*L* sample of cells, treated for various periods and/or concentrations (of the different forms of mercury being studied), was mixed with 100 μ*L* of 0.4% (w/v) aqueous trypan blue, and incubated at room temperature for one minute. The number of cells not excluding trypan blue was counted in hemacytometer using an inverted biological microscope (Hund Wetzlar).

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. The double encapsulated target was sent to the Missouri University Research Reactor (MURR) facility (Columbia, MO) to be irradiated (by neutron activation) for 4 wk. The irradiated target was placed in protected storage for 10 d 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 4 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 Hg using plasma-coupled elemental mass spectrometry. The radioactivity of the solution was determined with a Wallac (Gaithersburg, MD) Wizard 3″ 1480 automatic gamma counter (^{203}Hg counting-efficiency ~ 50%). The specific activities of the $^{203}Hg^{2+}$ used in the present study ranged between 8 and 9 mCi/mg Hg.

Statistical Analysis

Data are expressed as mean ± SE, unless otherwise stated. Since data expressed as a percent of a total do not fit a normal or Gaussian distribution, all data expressed as a percent were first normalized using the arcsine transformation prior to applying any parametric statistical analysis. This transformation takes the arcsine of the square root of the decimal fraction of the percent score.

Differences between means for each parameter studied were assessed statistically using a three-way analysis of variance (ANOVA) followed by the Tukey's protected *t*-test, which is a post hoc test that allows for the statistical comparisons between all logical combinations of any two means. The level of significance for all statistical analyses was chosen a priori to be $p < .05$.

RESULTS

Kinetic Analysis of Sodium-Dependent and Sodium-Independent Transport of Cysteine or Homocysteine S-Conjugates of Hg^{2+}

During 30 min of exposure, uptake of Hg^{2+} in the monocytes exposed to Cys-Hg-Cys or Hcy-Hg-Hcy was significantly greater, at each concentration studied, in the presence of 140 m*M* NaCl than in the presence of 140 m*M* NMDG (Figures 1 and 2). The 30-min period was chosen for study because we have evaluated previously kinetic parameters in various cell

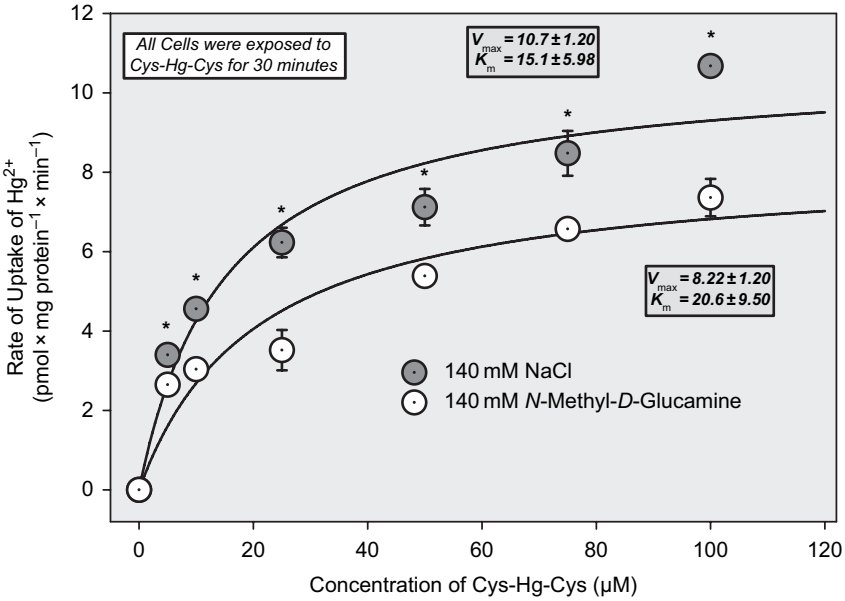

FIG. 1. Concentration-dependent uptake and accumulation of inorganic mercury (Hg^{2+}) in cultured RAW cells exposed to Cys-Hg-Cys for 30 min, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < 0.05$) from the mean for the corresponding group of RAW cells exposed to Cys-Hg-Cys in the presence 140 m*M* *N*-methyl-*D*-glucamine.

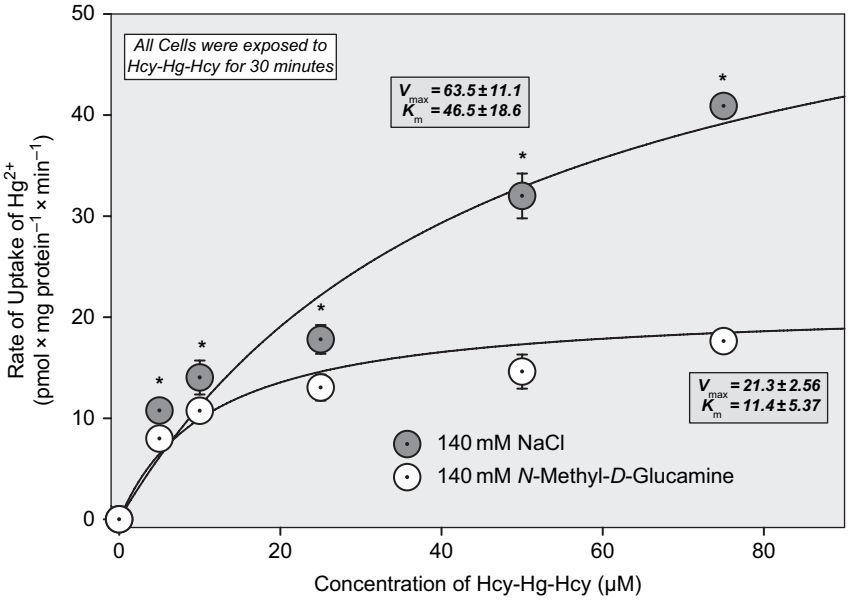

FIG. 2. Concentration-dependent uptake and accumulation of inorganic mercury (Hg^{2+}) in cultured RAW cells exposed to Hcy-Hg-Hcy for 30 min, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to Hcy-Hg-Hcy in the presence 140 m*M* *N*-methyl-*D*-glucamine.

lines during this same time. Unfortunately, under the conditions of the present investigation, the time-dependent uptake of Hg^{2+} during the initial 30 min fell slightly outside of the linear range (Figures 3 and 4), which may have affected kinetic parameters.

The estimated K_m for the uptake of Hg^{2+} in the cells exposed to Cys-Hg-Cys in the presence of NaCl was 15.1 ± 5.98 μ*M*, while the K_m for the uptake of Hg^{2+} in the cells exposed to Cys-Hg-Cys in the presence of 140 m*M* NMDG was greater at 20.6 ± 9.50 μ*M*. Similarly, the greater V_{max} for

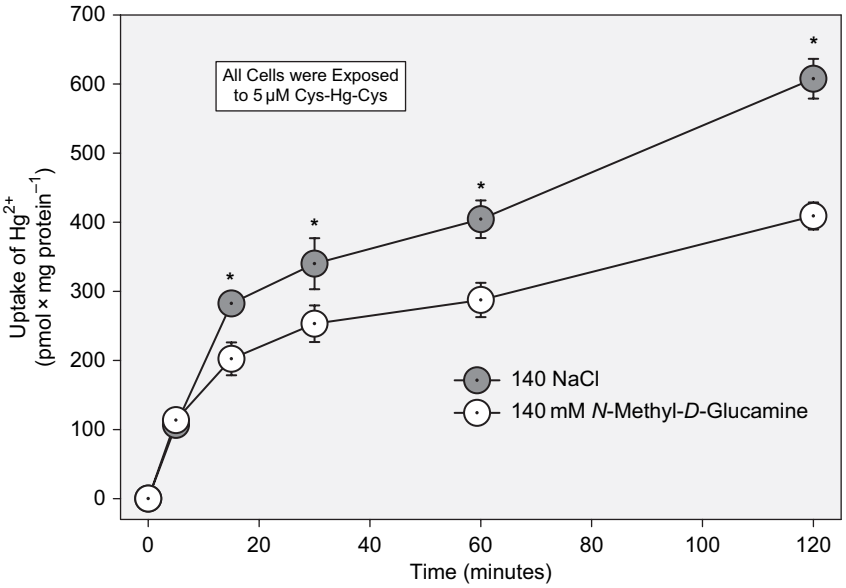

FIG. 3. Time-dependent uptake and accumulation of inorganic mercury (Hg^{2+}) in cultured RAW cells exposed to 5 µM Cys-Hg-Cys, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to Cys-Hg-Cys in the presence 140 m*M* *N*-methyl-*D*-glucamine.

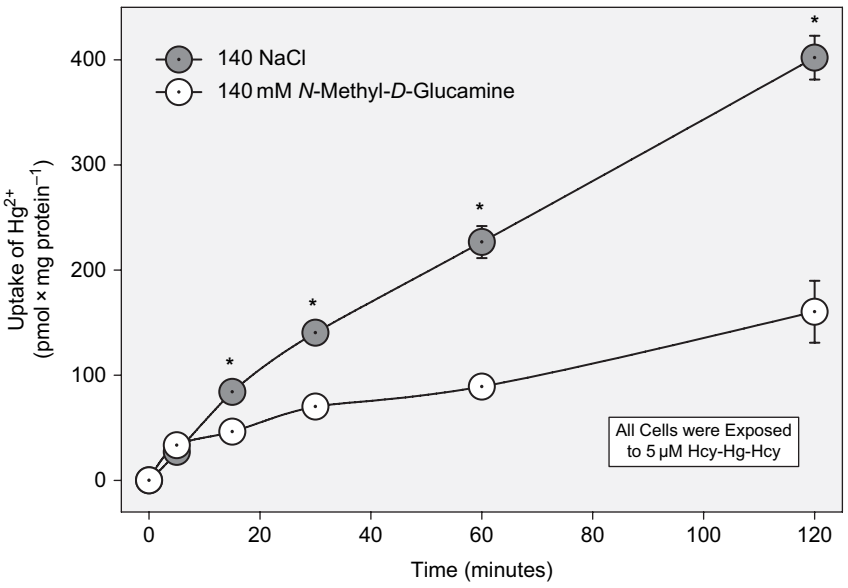

FIG. 4. Time-dependent uptake and accumulation of inorganic mercury (Hg^{2+}) in cultured RAW cells exposed to 5 µM Hcy-Hg-Hcy, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to Hcy-Hg-Hcy in the presence 140 m*M* *N*-methyl-*D*-glucamine.

the uptake of Hg^{2+} as Cys-Hg-Cys in the present of NaCl was 10.7 ± 1.20 pmol × mg protein^{-1} × min^{-1}. By contrast, the V_{max} of 8.22 ± 1.20 pmol × mg protein^{-1} × min^{-1} for the uptake of Hg^{2+} as Cys-Hg-Cys in the presence of 140 m*M* NMDG was lower than that at in the presence of Na.

Kinetic analyses indicate that the rates of uptake of Hg^{2+} tended to be greater in RAW cells exposed to Hcy-Hg-Hcy than in RAW cells exposed to Cys-Hg-Cys (Figures 1 and 2). In the cells exposed to Hcy-Hg-Hcy, the K_m for the uptake of Hg^{2+} was greater in the presence of 140 m*M* NaCl ($K_m = 46.5 \pm$

18.6) than in the presence of 140 m*M* NMDG (K_m = 11.4 ± 5.37). On the other hand, the V_{max} in the cells exposed to Hcy-Hg-Hcy in the presence of 140 m*M* NaCl (63.5 ± 11.1 pmol × mg protein^{-1} × min^{-1}) was greater than that in the cells exposed to Hcy-Hg-Hcy in the presence of 140 m*M* NMDG (63.5 ± 11.1 pmol × mg protein^{-1} × min^{-1}).

Time-Dependent Analysis of Sodium-Dependent and Sodium-Independent Transport of Cysteine or Homocysteine S-Conjugates of Hg^{2+}

Assessment of the uptake of Hg^{2+} as the mercuric conjugates, Cys-Hg-Cys and Hcy-Hg-Hcy, over various times revealed that there is time-dependent uptake of these species of mercury (Figures 3 and 4). In addition, the data show that the uptake of Hg^{2+} in the cells exposed to Cys-Hg-Cys or Hcy-Hg-Hcy was significantly greater in the presence of 140 m*M* NaCl than in the presence of 140 m*M* NMDG as early as 15 min of exposure.

Temperature-Dependent Uptake of the Cysteine S-Conjugates of Hg^{2+}

At 37°C, uptake of Hg^{2+} in the RAW cells exposed to Cys-Hg-Cys (in the presence of 140 m*M* NaCl) occurred in a concentration-dependent manner, similar to that demonstrated in Figure 1. By contrast, the uptake of Hg^{2+} in the RAW cells exposed to Cys-Hg-Cys at 4°C was negligible to very low over the course of the concentrations Cys-Hg-Cys studied (Figure 5).

Kinetic Analysis of Sodium-Dependent and Sodium-Independent Transport of Cysteine or Homocysteine S-Conjugates of CH_3Hg^+

Over 15 min of exposure, uptake of CH_3Hg^+ in the monocytes exposed to CH_3Hg-Cys or CH_3Hg-Hcy was substantial, particularly at the higher concentrations of CH_3Hg-Cys and CH_3Hg-Hcy studied. Interestingly, there was no significant difference in the uptake of CH_3Hg^+ between the cells treated in the presence of 140 m*M* NaCl and the cells exposed to 140 m*M* NMDG at each concentration studied, with only one exception in the cells treated with CH_3Hg-Hcy (Figures 6 and 7).

In the cells exposed to CH_3Hg-Cys in the presence of NaCl, the estimated K_m for the uptake of Hg^{2+} was 862 ± 217 μ*M*. Moreover, the K_m for the uptake of CH_3Hg^+ in the cells exposed to CH_3Hg-Cys in the presence of 140 m*M* NMDG was 897 ± 103 μ*M*. The V_{max} for the uptake of CH_3Hg^+ as CH_3Hg-Cys, in the present of 140 m*M* NaCl was 2308 ± 330 pmol × mg protein^{-1} × min^{-1}. In the presence of 140 m*M* NMDG, the V_{max} for the uptake of CH_3Hg^+ as CH_3Hg-Cys was 2224 ± 147 pmol × mg protein^{-1} × min^{-1}.

As was the case Hg^{2+}, kinetic analyses indicate that the rates of uptake of CH_3Hg^+ tended to be greater in RAW cells exposed to Hcy conjugate of CH_3Hg^+ than the Cys conjugate of CH_3Hg^+ (Figures 6 and 7). In the RAW cells exposed to CH_3Hg-Hcy, the K_m for the uptake of CH_3Hg^+ was 1873 ± 165 μ*M* in the presence of 140 m*M* NaCl and 667 ± 48.5 μ*M* in the presence of 140 m*M* NMDG. The V_{max} in the cells exposed to CH_3Hg-Hcy was 4268 ± 270 pmol × mg protein^{-1} × min^{-1} in the presence of 140 m*M* NaCl and 2380 ± 90.2 pmol × mg protein^{-1} × min^{-1} in the presence of 140 m*M* NMDG.

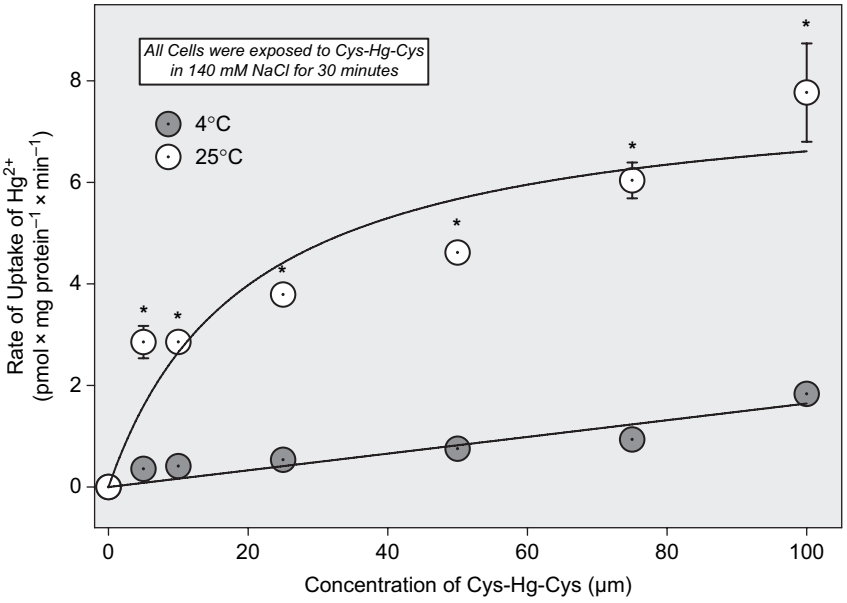

FIG. 5. Effect of temperature on the concentration-dependent uptake and accumulation of inorganic mercury (Hg^{2+}) in cultured RAW cells exposed to Cys-Hg-Cys for 30 min, in the presence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to Cys-Hg-Cys at 4°C.

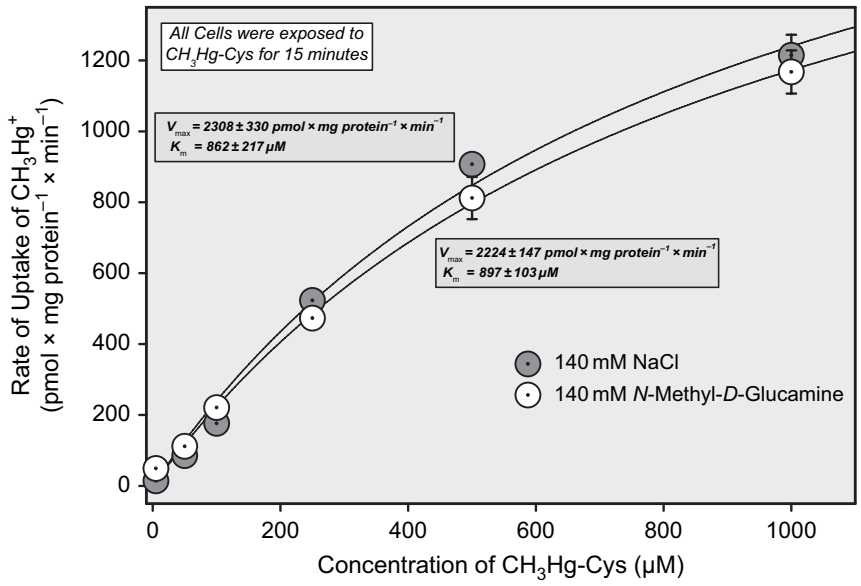

FIG. 6. Concentration-dependent uptake and accumulation of methylmercury (CH_3Hg^+) in cultured RAW cells exposed to CH_3Hg-Cys for 15 min, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations.

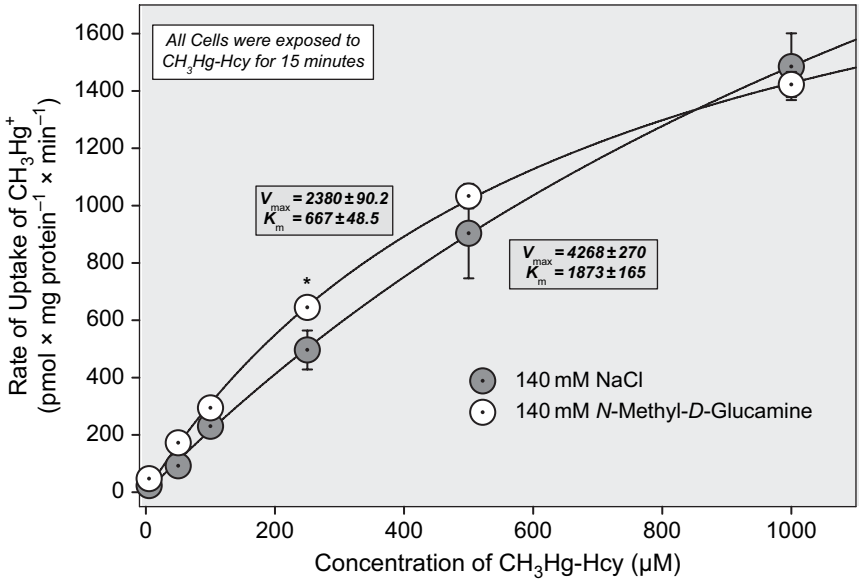

FIG. 7. Concentration-dependent uptake and accumulation of methylmercury (CH_3Hg^+) in cultured RAW cells exposed to CH_3Hg-Hcy for 15 min, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to CH_3Hg-Hcy in the presence 140 mM *N*-methyl-*D*-glucamine.

Time-Dependent Analysis of Sodium-Dependent and Sodium-Independent Transport of Cysteine or Homocysteine S-Conjugates of Hg^{2+}

Although the patterns of uptake were somewhat irregular, time-dependent uptake of CH_3Hg^+ were demonstrated in the RAW cells exposed to the methylmercuric conjugates, CH_3Hg-Hcy (Figure 8) and CH_3Hg-Hcy (Figure 9). However, only in the cells exposed to CH_3Hg-Cys were significant differences detected between corresponding groups of cells treated in the presence of 140 m*M* NaCl and those treated in the presence of 140 m*M* NMDG (Figure 8).

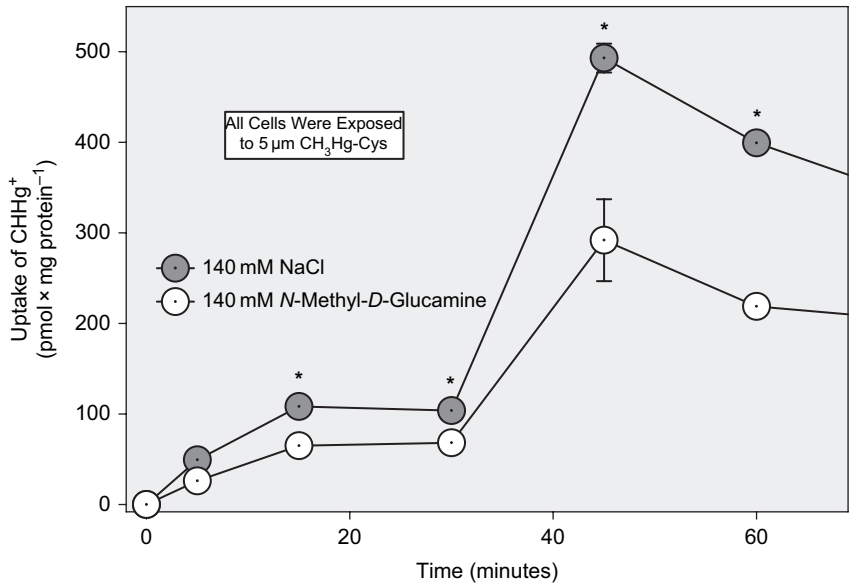

FIG. 8. Time-dependent uptake and accumulation of methylmercury (CH_3Hg^+) in cultured RAW cells exposed to 5 µM CH_3Hg-Hcy, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to CH_3Hg-Hcy in the presence 140 mM *N*-methyl-*D*-glucamine.

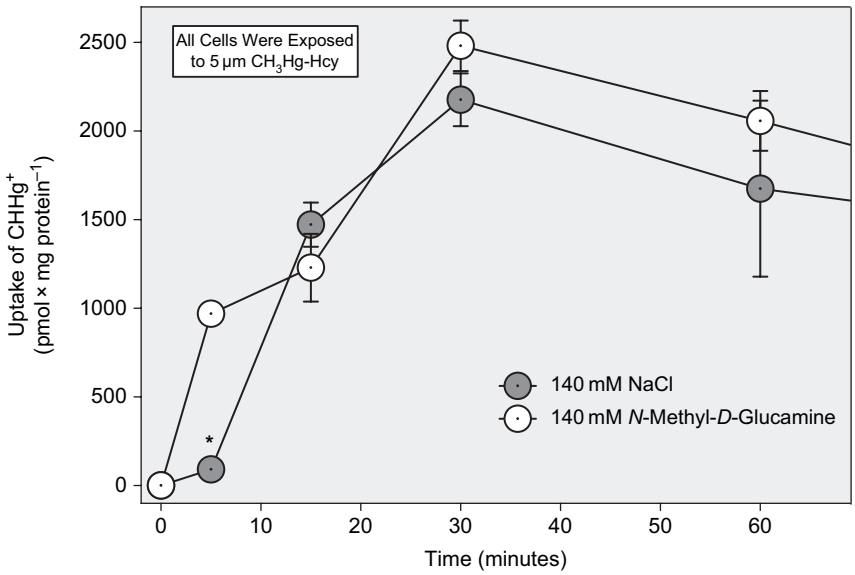

FIG. 9. Time-dependent uptake and accumulation of methylmercury (CH_3Hg^+) in cultured RAW cells exposed to 5 µM CH_3Hg-Cys, in the presence or absence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to CH_3Hg-Cys in the presence 140 m*M* *N*-methyl-*D*-glucamine.

Temperature-Dependence Uptake of the Cysteine S-Conjugates of Hg^{2+}

As was the case with Hg^{2+}, uptake of CH_3Hg^+ in the raw cells exposed to CH_3Hg-Cys at 37°C (in the presence of 140 m*M* NaCl) occurred in a concentration-dependent manner (Figure 10). However, the uptake of CH_3Hg^+ in the RAW cells exposed to CH_3Hg-Cys at 4°C was negligible over the course of the concentrations CH_3Hg-Cys studied (Figure 10).

DISCUSSION

Monocyte/macrophages are potentially important cellular targets of mercury because of their role in inflammatory and immune responses. In tissue macrophages arising from peripheral blood monocytes, there is evidence indicating that mercuric species can enter these cells by a mechanism involving endocytosis. Approximately 70% of the mercury taken up by macrophages appears to involve the uptake of

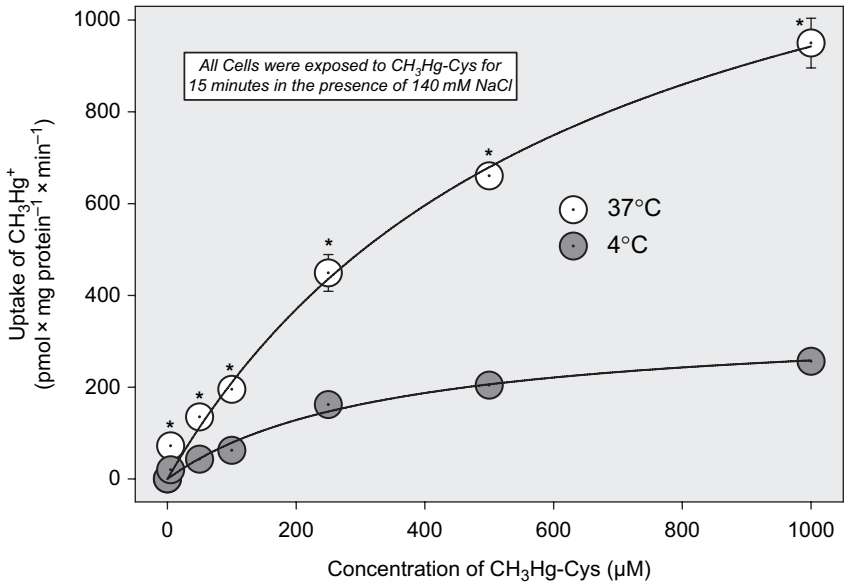

FIG. 10. Effect of temperature on the concentration-dependent uptake and accumulation of methylmercury (CH_3Hg^+) in cultured RAW cells exposed to CH_3Hg-Cys for 15 min, in the presence of 140 m*M* NaCl. Each value represents the mean ± SE of 3–4 observations. Asterisk indicates significantly different ($p < .05$) from the mean for the corresponding group of RAW cells exposed to CH_3Hg-Cys at 4°C.

mercury-containing particles by pinocytosis (Cunha et al., 2004: Cherdwongcharoensuk et al., 2002). Several investigators have demonstrated that when certain metal species, including those of mercury, are taken up by monocytes/macrophages, they alter significantly metabolic activity in the cells (Wataha et al., 2002; Noda et al., 2002). At extracellular concentrations as low as 75–300 n*M*, mercuric species have been shown to alter significantly mitochondrial activity and induce increases in cytosolic levels of glutathione (GSH) in monocytes (Messer et al., 2005). Moreover, the ability of monocytes (THP-1 cells) to proliferate decreases over periods of prolonged exposure to mercuric species.

The greatest level of uptake of Hg^{2+} and CH_3Hg^+ by monocytes in vivo presumably occurs shortly after exposure, when plasma level of these forms of mercury are highest. Normally, plasma levels of mercury decline rapidly during the initial several hours after exposure due to the initial phase of distribution to other organs in the body, especially in the kidneys and liver. With repeated exposures, however, it is more likely that plasma levels would be maintained at much higher levels, increasing the probability of monocytes, and other cellular components of blood, taking up even greater levels of these species of mercury.

Mercury also appears to have profound effects on lymphocytes. Recent data from mouse thymocytes exposed to heavy metals such as mercury, cadmium, arsenic, gold, lead, or manganese show that mercury was the only metal that consistently induced toxic effects in these cells (Mondal et al., 2005). Moreover, cellular intoxication by mercury was increased in the presence of 2-metacaptoethanol (2-ME), which apparently enhanced the cellular uptake of inorganic mercury (Hg^{2+}) by 10- to 20-fold. It was suggested that relatively noncytotoxic concentrations of Hg^{2+} might disrupt peripheral tolerance, leading to autoimmune disease. MacCabe and colleagues have suggested that Hg^{2+} modulates several signaling pathways in lymphocytes (McCabe et al., 2005). Based on these findings, they suggested that this may serve as a mechanism that deregulates the immune response.

Until the present study, very little was known about the potential for membrane transport proteins to be involved in the uptake of mercuric species in monocytes or macrophages. It was the findings from studies on the transport mercuric species in specific types of epithelial cells that led us to the present study. The findings from these studies show clearly that thiol S-conjugates of Hg^{2+} and CH_3Hg^+ are species of mercury that are taken up by carrier-mediated transport (Aslamkhan et al., 2003; Bridges & Zalups, 2004; Bridges et al., 2004; Canon et al., 2000, 2001; Mokrzan et al., 1995; Simmons-Willis et al., 2002; Zalups, 2000; Zalups & Ahmad, 2004, 2005; Zalups et al., 2004). For example, we have demonstrated previously in transfected Madin–Darby canine kidney (MDCK) cells and in oocytes from *Xenopus laevis* that the amino acid transporter system $b^{0,+}$, which is present on the luminal plasma membrane of renal proximal tubular epithelial cells, is capable of transporting Cys and Hcy S-conjugates of Hg^{2+} (Bridges et al., 2004; Bridges & Zalups, 2004). We have also demonstrated that the uptake Cys-Hg-Cys and Hcy-Hg-Hcy by system $b^{0,+}$ occurs by a mechanism involving molecular mimicry, whereby

these mercuric species mimic the disulfide-containing amino acid cystine, which is a natural substrate of this amino acid transporter. In another series of recent studies, we demonstrated that the organic anion transporters OAT1 and OAT3, which are present on the basolateral plasma membrane of renal proximal tubular epithelial cells, are capable of transporting Cys and Hcy S-conjugates of both Hg^{2+} and CH_3Hg^+ (Zalups & Ahmad, 2004, 2005; Zalups et al., 2004). Additional findings from Simmons-Willis et al. (2002) indicate that the Na-independent transport system L, which is present in several types of epithelial cells, is also capable of transporting the methylmercuric conjugate CH_3Hg-Cys, presumably as a molecular mimic of the amino acid methionine. Based on the findings from these studies, there is a distinct possibility that thiol S-conjugates of Hg^{2+} and CH_3Hg^+ may also gain entry into monocytes and macrophages by similar carrier-mediated mechanisms.

Data from the present study indicate that Cys or Hcy S-conjugates of Hg^{2+} or CH_3Hg^+ are mercuric species that capable of being transported by monocytes. Time-dependent experiments on the uptake of Hg^{2+} as Cys-Hg-Cys or Hcy-Hg-Hcy or CH_3Hg^+ as CH_3Hg-Cys or CH_3Hg-Hcy show that uptake increases over time, in both the presence and absence of sodium ions. Involvement of a membrane carrier protein in these processes is particularly supported by the findings on concentration dependence, sodium dependence, and by temperature dependence. Uptake of Cys-Hg-Cys and CH_3Hg-Cys occurred almost exclusively at 37°C, with negligible uptake at 4°C. Thus, nonspecific binding and uptake by diffusion are ruled out as mechanisms responsible for the uptake at 37°C. Kinetic analyses for the uptake of the S-conjugates show that the uptake of the different mercuric species fit Michaelis–Menten properties. Interestingly, the present findings indicate that distinct sodium-dependent and sodium-independent mechanism(s) are involved in the uptake of the S-conjugates of Hg^{2+} in monocytes. However, it appears that only one or more sodium-independent mechanisms are involved in the uptake of the S-conjugates of CH_3Hg^+. In terms of overall rates of uptake, profoundly greater levels of uptake of mercuric ions occur in monocytes when the mercuric ions are in the methylated form, as opposed to the divalent inorganic form.

Unfortunately, the present findings do not identify the type or types of carrier proteins involved in the uptake the mercuric species studied. There are, however, reports of transcriptional and translational expression of various transporters involved in monocytes. Amino acid transporters like system y^+L have been implicated in the uptake of L-arginine in human monocytes (Rotoli et al., 2004). Transporter proteins for lactate such as monocarbohydrate transporter 1, 2, and 4 (MCT1, MCT2 and MCT4) have also been shown to be present in monocytes and lymphocytes by Western blots (Merenzhinskaya et al., 2004). In addition to these transporters, various other membrane transport proteins are expressed in monocytes and macrophages. For example, the divalent-metal transporter NRAMP1 (also known as DMT1 or DCT1) and certain glutamate transporters, such as the excitatory amino acid transporters (EAAT1 and EAAT2) and system x_c^- (cystine/glutamate), are expressed in the macrophages under various conditions. Interestingly, blood-borne monocytes do not normally transport glutamate. However, when monocytes undergo activation following exposure to tumor necrosis factor (TNF) α and transform into macrophages, they begin to express the glutamate transporters (EAATs) and the cystine/glutamate antiporter (Rimaniol et al., 2000). Rimaniol et al. (2001) has shown that macrophages take up cystine through system x_c^-. Interestingly, freshly isolated macrophages do not express EAATs, but apparently cultured macrophages do, indicating that macrophages generate these transporters only under certain conditions, presumably when they are in a state of activation. It has also been shown that NRAMP1 functions as a divalent-metal efflux pump at the phagosomal membrane of macrophages and neutrophils (Forbes & Gros, 2001). Likewise, ABCG1 has been demonstrated to be responsible for cholesterol efflux in macrophages (Schachter, 2005).

In summary, the present findings demonstrate that Cys and Hcy S-conjugates of Hg^{2+} or CH_3Hg^+ are transportable substrates that are taken up by mouse monocytes. These findings provide substantial evidence indicating carrier mediated processes in the uptake of these mercuric species. Clearly, however, additional studies are warranted in order to identify the specific carrier proteins involved in the uptake of Cys and Hcy S-conjugates of Hg^{2+} or CH_3Hg^+ in monocytes and macrophages.

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