Effects of Methylmercury on Primary Brain Cells in Mono- and Co-culture

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We report on the uptake of MeHg in astrocytes and neurons, as well as specific indicators of neurotoxicity. Cerebellar granule neurons and astrocytes separately and in co-culture were cultured in the presence of MeHg and changes in 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT)-reduction, lactate dehydrogenase (LDH) leakage, and cellular content of glutathione and amino acids were used as indicators of MeHg toxicity. Mitochondria in cortical astrocytes were slightly more sensitive than those in cerebellar astrocytes to the toxic effects of MeHg; furthermore, cellular integrity was better preserved in cerebellar astrocytes. When neurons and astrocytes from cerebellum were incubated in separable co-cultures using inserts, the astrocytes showed cellular damage at lower exposure to MeHg while neurons showed less changes compared to respective cell types in mono-cultures. Mercury uptake studies at 25 μM MeHg (10% serum present) showed that for neurons in co-culture the uptake was 1/3 compared to mono-cultures. In contrast, for astrocytes in co-culture, uptake was increased by 75%. A MeHg concentration-dependent increase of glutamate content in mono-cultures was noted. When MeHg concentration was increased to 10, 25, or 50 μM, neurons in co-cultures decreased their glutamate content, whereas astrocytes showed an increase. Other amino acids, such as glutamine, serine, valine, isoleucine, taurine, and phenylalanine were unaffected by MeHg. Glutathione content showed MeHg concentration-dependent changes in astrocytes and was increased in neurons in co-culture incubated with 5 μM MeHg. In conclusion, astrocytes appear to increase neuronal resistance, indicating a possible protective role for astrocytes in MeHg neurotoxicity.

Key Words: methylmercury; astrocytes; neurons; glutamate; MTT; LDH; amino acids.

INTRODUCTION

Except for the dialkyl organomercurials, methylmercury (MeHg) represents the most toxic of the organic mercury compounds. MeHg is a potent fungicide and its past usage in crop protection has caused human poisonings. As a result of biotransformation of mercury compounds released from anthropogenic sources in waterways, MeHg-adulterated fish have also been proven to be a major source of human poisoning (for a review, see Clarkson [2002]). The neurotoxicity of organomercurial compounds may result from a number of interferences with critical processes in the cells, e.g., mitochondrial activity, cell membrane properties, or cytoskeletal integrity. These aspects have recently been reviewed by Castoldi et al. (2001) and Sanfeliu et al. (2003).

The mechanisms of MeHg neurotoxicity are unknown, and it is a particularly intriguing fact that a compound that readily reacts with any sulfhydryl group shows high organ specificity. Even within the brain, the neuronal loss is limited to certain areas while sparing others. A wide range of cell cultures have been used to investigate the mechanisms of MeHg-induced neurotoxicity. However, most studies use cell lines, which may lack features important to the question of cellular selectivity. In studies using primary cell cultures, both toxicological and metabolic effects of MeHg have been addressed in mono-cultures of either astrocytes or neurons. However, in the central nervous system (CNS) the astrocytes are in close proximity to neurons, and thus it is important to determine the consequences of MeHg exposure when both cell types are present. The co-culture system used in the present investigation is a suitable tool with which to study such interaction and potential detrimental effects of MeHg. It also has the advantage of using primary cells, whereas other co-culture systems that have used cell lines, which may confound the utility of the results (Toimela et al., 2004).

We have exposed the cell cultures for 24 h or less to MeHg concentrations ranging from 1 to 50 μM in the presence of 10% fetal calf serum (FCS). As MeHg will bind to any sulfhydryl group, the introduction of FCS will reduce the effective concentration toward the exposed cells, thus justifying higher concentrations of MeHg in the incubation mix compared to studies which use serum-free incubations. When examining cell cultures at a high dose of MeHg over prolonged periods of time, it is important to include cytotoxicity tests indicative of the degree of cellular damage caused by the compound. In the...
present study we included the 3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide (MTT)-assay as an indicator of mitochondrial activity and lactate dehydrogenase (LDH)-leakage as indicator of cell membrane integrity.

There is conflicting evidence regarding the preferential uptake of MeHg in neurons or astrocytes. Using a silver impregnation technique, Charleston et al. (1995) found that mercury rapidly accumulated in astrocytes. Magos et al. (1985), using principally the same technique, identified neurons as prime accumulators of mercury. However, the silver-impregnation technique will react only with Hg$^{2+}$, and thus the method does not reflect the distribution of organic mercury. We determined the 24-h MeHg uptake in mono- and co-cultures from cerebellum by measuring $^{14}$C-labeled MeHg. It has previously been shown that demethylation of MeHg in vivo is a rather slow process, taking several days in the intact rat (Magos et al., 1985; Syversen, 1974). We measured $^{14}$C-labeled MeHg as representing total mercury present in cells after the 24-h in vitro incubation.

Glutamate has dual characteristics, both as the major excitatory neurotransmitter and as a neurotoxin implicated in multiple neurodegenerative diseases (Blood et al., 1969; Fonnum, 1984; Olney et al., 1971; Rothman and Olney, 1986). The effect of glutamate is terminated by uptake into the surrounding cells, and astrocytes represent a major site for glutamate uptake (Gegelashvili and Schousboe, 1998). In astrocytes, glutamate is converted to glutamine and released for uptake by neurons (for a review, see Sonnewald et al. [1997]). Such interaction is very important for optimal neuronal function since there is a constant drain of tricarboxylic acid (TCA) cycle intermediates for neurotransmitter synthesis. Effects of MeHg on [U-13C]glutamate metabolism in cerebral and cortical astrocytes have been analyzed (Allen et al., 2001; Qu et al., 2003). Reduced glutamate uptake and glutamine synthesis was detected in cerebellar astrocytes, whereas MeHg had little effect on [U-13C]glutamate metabolism in cortical astrocytes. However, reduced glutamate uptake was demonstrated in cortical astrocytes by Aschner et al. (2000). Previous studies on the mechanism of MeHg neurotoxicity have implicated the generation of reactive oxygen species (ROS) and depletion of the intracellular pool of glutathione (GSH) as important contributors to observed MeHg-induced cytotoxicity (Sanfeliu et al., 2001).

The present study was undertaken to assess the effects of MeHg on neurons and astrocytes derived from selective brain regions, and to test the hypothesis that MeHg-induced neuronal toxicity can be attenuated under conditions where neurons are co-cultured in the presence of astrocytes.

**MATERIALS AND METHODS**

**Materials.** Cells were grown in 12-well plastic tissue culture plates purchased from Nunc A/S (Roskilde, Denmark) and Falcon (Becton Dickinson Labware). The inserts were purchased from Nunc A/S (polycarbonate membrane, 0.4 μm) and Falcon (polylethylene teraphlatel (PET) track-etched membrane, 0.4 μm). Fetal calf serum was from Seralab Ltd. (Sussex, UK) and culture medium from GIBCO BRL, Life Technologies (Roskilde, Denmark). NMRI mice were purchased from the Mollegaard Breeding Center (Copenhagen, Denmark). $^{14}$C-labeled MeHg (Cat. No. ARV1302) was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). MeHg (Cat. No 23308) was purchased from K&K Laboratories (Plainview, NY). All other chemicals were of the purest grade available and they were purchased from commercial sources.

**Cell cultures.** The experimental design and methodology were approved by the Norwegian University of Science and Technology Animal Care and Use Committee. Cerebellar astrocytes were cultured as described elsewhere (Hertz et al., 1989). Briefly, cerebella were removed from 7-day-old mice and passed through Nitex nylon netting (80 μm pore size) into Dulbecco’s minimal essential medium (DMEM) containing 20% (v/v) fetal calf serum (FCS). The cell suspension was plated at a density of four cerebella per 12-well culture plate. Medium was changed two days after plating and twice a week thereafter, gradually decreasing the FCS concentration to 10%. At 14 days in culture, dibutyryl-cAMP was added to the medium for 1 week to promote the morphological differentiation of astrocytes. Experiments were performed on 3-week-old cultures. Cortical astrocytes were prepared from 1-day-old mice following the same procedure used for cerebellar astrocytes (Hertz et al., 1989) plated at a density of two cortices per 12-well culture plate.

Cerebellar neurons were isolated and cultured from the cerebellum of 7-day-old mice, after mild trypsinization of the tissue followed by trituration in a DNase solution containing a trypsin inhibitor derived from soybeans (Schousboe et al., 1989). Cells were suspended in a slightly modified DMEM containing 50 μM kainic acid and 10% (v/v) FCS and plated at density 2 × 10⁶ cells per well in a 12-well culture plate. The wells had been coated with poly-L-lysine. Cytosine arabinoside (20 μM) was added after 48 h to prevent astrocyte proliferation. Cells were used for experiments after 1 week in culture.

For the co-cultures, the neurons and astrocytes were prepared separately and seeded as described earlier. The astrocytes were cultured on inserts for 21 days, and the neurons were cultured in culture plates for 7 days prior to incubation in co-culture. The astrocytes were grown on permeable membranes in tissue culture plate inserts, and the neurons were cultured in standard tissue culture plates. The inserts were transferred to the neuronal plates 24 h prior to incubation with MeHg. At time of insert transfer, half of the neuronal medium was extracted and replaced with astrocyte media.

**MeHg treatment.** MeHg was dissolved in warm 5 mM Na₂CO₃ to make a 1 mM stock solution. From the stock solution, appropriate work dilutions were made in DMEM growth medium containing 10% (v/v) fetal bovine serum (FCS). For the MeHg uptake studies, a standard amount of $^{14}$C-labeled MeHg was added the solutions. The MeHg was added to the growth medium at the start of incubation. To maintain the correct hydrostatic pressure across the insert membrane, volumes in the co-cultures were regulated such that 15% of the total incubation volume was inside the insert and 85% was in the well. The MeHg was added to the growth medium at the start of incubation.

**MTT-test.** Acute cytotoxicity was evaluated on the mono-cultures and co-cultures exposed to MeHg using the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide) reduction assay modified after Dahlin et al. (1999) and Carmichael et al. (1987). Cellular viability was determined as percent of activity in control wells.

**LDH.** Lactate dehydrogenase (LDH)-leakage into the medium was measured as described by Bergmeyer (1972) using a Cobas Mira S analyzer. Leakage of LDH to the medium was expressed as a percentage of the total cellular LDH-activity as determined by disrupting the cells by incubation with 50 μl 1% sodium azide.

**Extraction.** At the end of the experiments, the medium was removed and cells were washed with 0.9% saline and extracted with 70% ethanol (v/v), followed by centrifugation at 4000 × g for 10 min. The supernatants and media...
were lyophilized and stored at −20°C. Cellular protein in the ethanol pellets was determined after re-dissolving in 1 M KOH at 37°C for 30 min, using the Pierce BCA (bicinchoninic acid) protein assay with bovine serum albumin as standard.

**HPLC.** Amino acids in the cell extracts were quantified by high performance liquid chromatography (HPLC) analysis on a Hewlett Packard 1100 system (Agilent, Palo Alto, CA) with fluorescence detection, after derivatization with o-phthalaldehyde and were separated on a ZORBAX SB-C18 (4.6 × 250 mm, 5 mm) column from Agilent with 50 mM sodium acetate buffer (pH 7.0) and methanol as eluents.

**Data analysis.** All results are given as mean ± standard deviation. Differences between groups were analyzed statistically with either the unpaired two-tailed Student’s t-test or one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) post hoc test for multiple comparison; p < 0.05 was considered statistically significant.

**RESULTS**

**Methylmercury Uptake**

As can be seen from Figure 1, neurons in mono-culture accumulate MeHg faster and to a greater extent than the astrocytes, in particular at high MeHg exposure. When uptake is measured in neuronal mono cultures there seems to be a threshold for uptake between 10 and 25 µM MeHg. The same can be observed for co-cultures with a threshold for uptake in astrocytes between 25 and 50 µM MeHg. When the neurons are co-cultured with astrocytes the neuronal MeHg uptake becomes roughly 1/3 compared to the mono-cultures. Concomitant with the observed change for neurons there is an increased uptake of MeHg in the astrocytes. There are significant differences in Hg uptake between mono- and co-cultures for both neurons and astrocytes at all concentrations at or above 5 µM MeHg.

**MTT**

Cerebellar neurons were exquisitely more sensitive to MeHg than astrocytes were, as measured by reduction of mitochondrial activity (Fig. 2). From the data presented in Figure 2, we can interpolate the EC50 (effective concentration for 50% inhibition), which is represented in Table 1. From Table 1 it can be seen that neurons in co-culture tolerate an almost fourfold greater MeHg concentration compared to the monocultures. On the other hand, astrocytes in co-culture tolerate less MeHg than in mono culture, and this likely reflects the increased uptake of MeHg in astrocyte co-cultures as shown in Figure 1. Figure 2 shows an initial deterioration followed by improvement in MTT activity in astrocyte mono-cultures when exposed to 1–25 µM MeHg. This may be an example of paradoxical upregulation of cellular function when exposed to a toxic material like MeHg. The cortical astrocytes are somewhat more sensitive to MeHg than the cerebellar astrocytes (Table 1).

**LDH**

The measurement of LDH leakage to the media of mono- and co-cultures of astrocytes and neurons confirmed the principal differences between the cell types as demonstrated by the MTT assay. When measured in mono-cultures, the cerebellar neurons had a significantly higher LDH leakage than either the cerebellar or the cortical astrocytes (Fig. 3). When exposed in co-culture with astrocytes, the neuronal LDH leakage decreased while the opposite effect occurred in the astrocytes.

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**FIG. 1.** Cellular mercury uptake was determined with 14C-labeled methylmercury chloride. Results are expressed as mean ± standard deviation (n = 3). *Significant difference (Student’s t-test) between mono- and co-cultures at p < 0.05 for cerebellar neurons. #The same significance, but for cerebellar astrocytes.

**FIG. 2.** Reduction of MTT was measured in mono- and co-cultures of cerebellar neurons and astrocytes after MeHg exposure for 24 h. Results are expressed as mean ± standard deviation (n = 6 for mono-cultures and n = 4 for co-cultures). *Significant difference (Student’s t-test) between mono- and co-cultures at p < 0.05 for cerebellar neurons. #The same significance, but for cerebellar astrocytes.
These data agree well with the uptake study, where the results show that astrocytes accumulate more MeHg in co-culture. An opposite trend is inherent in the neurons (Fig. 1).

**Glutamate Concentration**

From Figure 4 (A–D) it can be seen that the glutamate concentration was highest in neurons in mono-culture and that it decreased when these cells were cultured in the presence of astrocytes. Glutamate concentrations in astrocytes were independent of the culturing conditions, namely, the presence of astrocytes in mono or co-culture (black bars in Fig. 4 A, B). In the presence of 1 µM MeHg, astrocytes in mono-culture had increased glutamate concentration (Fig. 4 A), whereas neurons in mono- and co-culture showed increased glutamate

### TABLE 1

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<tr>
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<th>Mono-culture</th>
<th>Co-culture</th>
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<tr>
<td>Cerebellar neurons</td>
<td>2.2 ± 0.8</td>
<td>9.0 ± 1*</td>
</tr>
<tr>
<td>Cerebellar astrocytes</td>
<td>39.5 ± 1</td>
<td>34.5 ± 2*</td>
</tr>
<tr>
<td>Cortical astrocytes</td>
<td>33.0 ± 2**</td>
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*Note:* Values obtained by interpolation of dose–response curves. Each cell type was incubated alone or in co-culture comprised of cerebellar neurons and astrocytes. Values represent mean ± standard deviation. Experiments were conducted in six replicates for cerebellar monocultures and four for cocultures and four mono-cultures of cortical astrocytes.

*<i>p < 0.05</i> different from mono-culture.

**<i>p < 0.05</i> different from cerebellar astrocytes.

(Fig. 3). These data agree well with the uptake study, where the results show that astrocytes accumulate more MeHg in co-culture. An opposite trend is inherent in the neurons (Fig. 1).

**FIG. 3.** Lactase dehydrogenase leakage was measured in mono- and co-cultures of cerebellar neurons and astrocytes after MeHg exposure for 24 h. Results are expressed as mean ± standard deviation (n = 6 for mono-cultures and n = 4 for co-cultures) of percentage of total cellular LDH. *Significant difference (Student’s t-test) between mono- and co-cultures at p < 0.05 for cerebellar. #The same significance, but for cerebellar astrocytes.

**FIG. 4.** Amino acid concentration (nmol/ml) in cell extracts from cerebellar astrocytes in mono-culture (A) and co-culture (B). Likewise for neurons in mono-culture (C) and co-culture (D) in the presence of 0 (control, black column), 1 (white column), or 5 (black and white column) µM MeHg. Cells were cultured as described in Materials and Methods. Phe, phenylalanine. Statistical analysis between mono- and co-culture was performed with unpaired two-tailed Student’s t-test, p < 0.05 was considered statistically significant. Results are expressed as mean ± standard deviation (n = 6). Analyses between 0, 1, and 5 µM MeHg groups was performed using one-way ANOVA followed by the LSD (least significant difference) post hoc test, and p < 0.05 was considered statistically significant. *Significantly different from control. *Significantly different from 1 µM group. #Significantly different from mono-culture of under same incubation conditions.
concentration at 5 µM MeHg but not at 1 µM (Fig. 4 C, D). Glutamate concentrations in astrocytes in co-culture were unaffected by MeHg at 1 or 5 µM. When the MeHg concentration was increased to 10, 25, and 50 µM, neuronal co-cultures decreased their glutamate content, whereas astrocytes in co-culture showed an increase (Fig. 5).

Other Amino Acids and Glutathione Concentrations

Astrocytes in mono- (Fig. 4A) and co-cultures (Fig. 4B) were very similar in amino acid content; only glutathione (GSH) was decreased in astrocytes in co-cultures compared to mono cultures. However, GSH, glutamate, and taurine were increased in the presence of MeHg compared to controls in mono-cultures. Neurons in co-culture (Fig. 4D) showed a decrease in GSH, aspartate, taurine, alanine, and GABA compared to mono-cultures (Fig. 4 C) under all conditions except at 5 µM MeHg, where GABA concentration was the same in mono- and co-cultures. Glutamine concentration was decreased in neurons in mono-culture at 5 µM MeHg, but it remained unchanged otherwise. Glutathione was increased in neurons in co-culture incubated in the presence of 5 µM MeHg.

FIG. 5. Glutamate concentration represented as percentage of control (no MeHg) in cell extracts from cerebellar neurons and astrocytes in mono- and co-culture in the presence of 10, 25, or 50 µM MeHg. Cells were cultured as described in Materials and Methods. Results are expressed as mean and represent minimum and maximum values (n = 2).

DISCUSSION

The present data strongly suggest that cerebellar neurons accumulate more MeHg and at a faster rate than their companion astrocytes. There are significant differences between laboratories in the methodologies applied for determination of MeHg content in biological materials. Early reports on regional distribution of MeHg were based on autoradiography using the gamma emitter $^{203}$Hg. In such studies, the image resolution was insufficient to allow precise association of the label with a specific cell type (Chang and Hartmann, 1972). The autometallographic method (Danscher and Møller-Madsen, 1985; Danscher and Schröder, 1979) utilizes an excess of silver to create a silver deposit in the presence of Hg$^{2+}$. However, this technique does not detect organic forms of mercury, a fact that has been noted by, e.g., Evans et al. (1977) and Charleston et al. (1995), both of whom reported selective accumulation of Hg$^{2+}$ in astrocytes by the autometallographic method. Using the same method, however, Magos et al. (1985) found Hg$^{2+}$ to preferentially accumulate in neuronal cytoplasm of cerebellar Purkinje cells and to be totally absent from cerebellar granule cells, where the most prominent morphological changes were observed in response to MeHg exposure. In the present study, assuming that demethylation does not occur during the short incubation period of 24 h, we measured MeHg by detecting the $^{14}$C-isotope. Previous studies have shown that such demethylation reflects a rather slow process in brain tissue (Magos et al., 1985; Syversen, 1974). The large differences in MeHg accumulation between the different cell types and exposure conditions occur at very high exposures (>25 µM MeHg).

The MTT- and LDH-release assays indicated that cytotoxicity occurred at MeHg exposure levels of less than 1 µM, and neurons were significantly more sensitive than astrocytes. At 5 µM MeHg, almost 70% of the neuronal content of LDH had been released into the medium while the MTT-activity was reduced to 20% of control. This indicates severe damage to the cell membranes, as well as to mitochondria. The response to combining the cells in a co-culture suggests that the astrocytes protect the neurons and at the same time the astrocytes become more sensitive to the effects of MeHg. This conclusion strengthens earlier proposals that astrocytes play an important role in MeHg neurotoxicity (Aschner et al., 1994).

Cortical and Cerebellar Astrocytes

Differences between cortical and cerebellar astrocytes in response to MeHg have been reported elsewhere (Allen et al., 2001; Qu et al., 2003). In cerebellar astrocytes, the percentage of [U-$^{13}$C]glutamate used for energy production was decreased in the presence of 25 or 50 µM MeHg for 4 h, indicating selective mitochondrial vulnerability from the inhibitory effect of MeHg (Qu et al., 2003). Cortical astrocytes were exposed to either 1 µM for 24 h, or 10 µM for 30 min. The only difference from control was in the formation of [U-$^{13}$C]lactate from [U-$^{13}$C]glutamate, which was decreased in the 10 µM MeHg group. Also in the present study, differential vulnerability was detected with regard to mitochondrial functioning. MTT activity was decreased in cortical astrocytes at lower MeHg concentrations than in cerebellar astrocytes, whereas LDH leakage was more pronounced in the cerebellar astrocytes.
Glutamate Concentration

It is interesting to note that the glutamate concentration was highest in neurons in mono-culture and decreased to less than 50% when these cells were cultured in the presence of astrocytes. Unaltered glutamate content was detected in astrocytes in mono or co-culture, indicating that the difference in glutamate concentration in neurons in co-culture was caused by decreased synthesis by neurons. It can be speculated that astrocytes release a factor that regulates glutamate synthesis in neurons.

In the presence of 1 μM MeHg for 24 h, astrocytes in monoculture showed increased glutamate content, whereas in the neurons (mono- and co-culture) this level of increase was observed only after exposure to 5 μM MeHg. This difference agrees with the hypothesis that astrocytes are more susceptible to MeHg-induced changes than neurons (Aschner et al., 1994, 1995). However, astrocytes in co-culture were unaffected by MeHg at 1 or 5 μM. The reason for the increased glutamate concentration in astrocytes in mono-culture could be a decrease in glutamine synthetase activity, which has been shown to occur in cortical astrocytes, though reportedly by mercuric chloride and not by MeHg (Allen et al., 2001). At 10 μM MeHg, both astrocytic and neuronal co-cultures had increased glutamate content, whereas the concentrations in monocultures were unchanged (astrocytes) or decreased (neurons). At 25 μM MeHg, astrocytes in co-cultures still had elevated glutamate, whereas the concentration of this amino acid in neurons was back to normal levels. When MeHg concentration was increased to 50 μM, all cells showed decreased glutamate content as a result of severe cell damage (as outlined above).

Glutathione

The GSH content in co-cultured neurons was lowest of all cultures examined, indicating that astrocytes have better access to the GSH precursor cysteine (Shanker et al., 2001). Interestingly, MeHg did not affect GSH content in neurons and astrocytes in mono-culture, and only 1 μM MeHg led to an increase in GSH concentration in astrocytes and a decrease in neurons when present in co-cultures. Reactive oxygen species (ROS) are known to mediate MeHg-induced neurotoxicity in multiple experimental models. For example, MeHg induces ROS formation both in vivo (rodent cerebellum) and in vitro (isolated rat brain synaptosomes) (Ali et al., 1992), as well as in cerebellar neuronal cultures, a hypothalamic neuronal cell line, and in mixed reaggregating cell cultures (Gasso et al., 2001; Park et al., 1996; Sarafian, 1999; Sorg et al., 1998). It has been suggested from in vitro studies that the upregulation of GSH synthetic capacity might be a sensitive biomarker for sub-chronic MeHg exposure (Thompson et al., 1999). The present studies suggest that even in the presence of an insult, and independent of culturing situation (mono- versus co-cultures), both cell types are able to maintain a fairly stable redox status, likely an attempt to combat ROS generation.

Other Amino Acids

Glutamine, the important player in the glutamate–glutamine cycle (Berl and Clarke, 1983; Sonnewald et al., 1997), was generally not affected by culture conditions or MeHg. The same was true for other amino acids such as serine, valine, isoleucine, leucine, and phenylalanine. Only a slight decrease in glutamine was observed in neurons in mono-culture in the presence of 5 μM MeHg. At higher MeHg concentration, the amounts of alanine, serine, and phenylalanine were decreased in cerebellar astrocytes (Qu et al., 2003). This was thought to be due to interactions of MeHg with system L-transporters of neutral amino acids (Broer, 2002). Whereas Qu et al. (2003) observed decreased amounts of taurine when cerebellar astrocytes were preincubated with 25 and 50 μM MeHg for 4 h, this was not the case in the present study. However, the amounts of taurine in neurons in co-culture were significantly lower than those in mono-culture. This difference may be consistent with the protective effects of astrocytes in the coculture, and a lesser need for intracellular taurine. Taurine is a neuroprotectant, and it has been established that osmoregulation and postischemic glutamate surge suppression (PIGSS) are among the important mechanisms in the neuroprotective properties of taurine suggested by Lo et al. (1998) and Khan et al. (2000).

In summary, the present study demonstrates that astrocytes do protect neurons from the toxic effects of MeHg, even when the two cell types are separated by a semipermeable membrane. The basis of such a protection might partially reflect changes in cellular MeHg uptake, but also in the metabolic properties of the two cell types.

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