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Comparison of alterations in amino acids content in cultured astrocytes or neurons exposed to methylmercury separately or in co-culture

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Abstract

Methylmercury (MeHg) is an environmental toxicant that induces enduring neuropsychological deficits in humans. Although the mechanisms associated with MeHg-induced neurotoxicity have not yet been fully elucidated, some lines of evidence point out to excitatory amino acids dyshomeostasis as an important outcome of MeHg exposure. The present study was designed to characterize the effects of MeHg on amino acid content in co-cultured astrocytes and neurons or in each cell type under solitary conditions. The results showed that glutamate concentrations significantly decreased in neurons, but not in astrocyte cultures exposed to 10 μ M MeHg. The decrease in neurons was fully reversed when these cells were co-cultured with astrocytes. The content of other amino acids (aspartate, alanine, glycine and serine) decreased upon exposure to 10 μ M MeHg in both neurons and astrocytes cultured in solitary conditions, although the effect was generally smaller in astrocytes than in neurons. However, the content of these amino acids in each of the cell types was indistinguishable from controls when co-cultures were treated with MeHg. Overall, the results indicate that astrocytes, which are more resistant to amino acid modulation by MeHg, can i) mitigate the effects of MeHg that occur in neurons cultured in solitary conditions and ii) become themselves more MeHg resistant in the presence of neurons. Delineating the mechanisms underlying the mutual neuroprotective effects of astrocytes and neurons in co-culture to MeHg-induced amino acid imbalance requires further investigation.

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Keywords

methylmercury; amino acids; astrocytes; neurons; co-culture

Introduction

Over the last several decades, the neurotoxicity of mercury (Hg) compounds has been the focus of many studies with a particular emphasis on the effects of methylmercury (MeHg). Most human exposures to this toxicant occur through food sources, primarily fish consumption (Airey 1983; WHO 1990; Björnberg et al., 2003). Multiple mechanisms have been implied in MeHg-induced neurotoxicity, including the inhibition of the uptake of amino acids (e.g., cysteine, glutamate), both in neurons and astrocytes (Shanker et al., 2001; Kaur et al., 2006). Several studies have demonstrated that MeHg inhibits astrocytic glutamate and aspartate uptake and stimulates their efflux, subsequently increasing the glutamate concentration in the extracellular fluid and sensitizing neurons to excitotoxic injury (Brookes and Kristt 1989; Aschner et al., 1990; 1993; Qu et al., 2003; Morken et al., 2005). MeHg also selectively inhibits the astrocytic transport of cysteine (and cystine), thereby altering intracellular redox status and glutathione (GSH) content (Allen et al., 2001a; Shanker and Aschner, 2003; Shanker et al., 2001). The *in vitro* co-application of non-toxic concentrations of mercury with glutamate induces the appearance of typical neuronal lesions found with excitotoxic stimulation (Matyja and Albrecht 1993). *In vivo* experiments also demonstrate that MeHg exposure results in increased extracellular fluid concentrations of glutamate, indicating that alteration of glutamate transmission is involved in MeHg-induced neurotoxicity (Juárez et al., 2002).

Astrocytes are the most numerous, non-neuronal cell types in the central nervous system (CNS) and perform several functions essential for normal neuronal activity, including glutamate uptake (Aschner et al., 1999). Astrocyte glutamate uptake is mediated by a Na⁺-dependent mechanism and is metabolized to glutamine by the astrocyte-specific enzyme, glutamine synthetase (GS) (Martinez-Hernandez et al., 1977). Astrocyte-derived glutamine is used by neurons for the re-synthesis of glutamate. This glutamate-glutamine pathway constitutes a major source of a glutamate pool in the brain (Berl et al., 1962). Several studies in primary astrocyte cultures have demonstrated that glutamate uptake is significantly attenuated in these cells upon exposure to MeHg (Aschner et al., 1990; 1993). Interference with glutamate transporter function results in increased extracellular glutamate concentrations, leading to glutamate excitatory damage. Corroborating this effect, *in vivo* studies confirm that damage associated with MeHg correlates to brain areas with dense glutamatergic innervation (Miyamoto et al., 2001).

In addition to the glutamate-glutamine pathway, other metabolic routes regarding neuron-glial interactions are important to maintain both metabolic and neurotransmitter homeostasis. Indeed, (i) glutamine from astrocytes is also essential for the synthesis of GABA in neurons (Albrecht et al., 2007); (ii) alanine, which can be transported from neurons to astrocytes through the alanine/lactate shuttle, acts as a substrate for oxidative metabolism in glutamatergic neurons (Peng et al., 1993); (iii) aspartate, which could be generated from transamination in a reaction involving glutamate, oxalacetate and the enzyme aspartate aminotransferase, acts as a glutamate co-transmitter in some neuronal pathways (Zhou et al., 1995), amplifying excitotoxicity through its action on NMDA receptors (La Bella and Piccoli, 2000); and (iv) glycine is supplied by astrocytes to neurons in the form of the dipeptide cysteinyl-glycine, which contributes to maintain intracellular GSH homeostasis (Dringen et al., 1999). Besides the important role of the aforementioned amino acids with respects to metabolism and neurotransmission in the CNS, there are no studies on the effects of MeHg toward glycine, alanine and serine. Moreover, although the effects of MeHg in neuronal and glial glutamate

homeostasis (Aschner et al., 1990; 2000; Allen et al., 2001b; Qu et al., 2003), as well as glial cystine (cystine) transport (Allen et al., 2001a; Shanker and Aschner, 2001) have been reported; these phenomena have not yet been explored from an interactive point of view involving both cell types.

Taking into account the importance of glial-neuronal interactions in maintaining amino acid metabolism/neurotransmission in the CNS, as well as the previous studies showing disruption of glutamate/cystine homeostasis in either neuron or astrocyte cultures under solitary conditions, the primary goal of this study was to examine the effects of co-culturing neurons and astrocytes on amino acid concentrations in these cells upon exposure to high (10 μ M) and low (1 μ M) MeHg concentrations. We hypothesized that the presence of astrocytes in the co-culture model would attenuate the MeHg-induced changes in amino acid levels inherent to neurons cultured under solitary conditions. Corroborating this hypothesis, we predicted that MeHg-induced changes in the neuronal levels of several amino acids would be fully reversed upon co-culturing with astrocytes.

Materials and Methods

Cell cultures

Astrocytic cultures from cerebral cortices of newborn (1-day-old) Sprague–Dawley rats were established as previously described (Yin et al., 2007). Briefly, rat pups were decapitated, and the cerebral cortices were removed. After the removal of the meninges, the cerebral cortices were digested with bacterial neutral protease (Dispase, Invitrogen, Carlsbad, CA, USA). Astrocytes were then recovered by the repeated removal of dissociated cells and plated in 6- or 12-well plates at a density of 1×10^5 or 4×10^4 cells/well. Twenty-four hours after the initial plating, the media were changed to preserve the adhering astrocytes and to remove neurons and oligodendrocytes. The cultures were maintained at 37°C in a 95% air/5% CO₂ incubator for 3 – 4 weeks in astrocyte growth medium composed of minimal essential medium (MEM) with Earle's salts supplemented with 10% heat-inactivated horse serum, 100 U/ml penicillin and 100 μ g/ml streptomycin. The medium was changed twice per week. These monolayer, surface-adhering cultures were >95% positive for the astrocytic marker, glial fibrillary acidic protein (GFAP).

Primary cultures of cortical neurons were obtained from 17- to 18-day-old fetal Sprague–Dawley rats. Briefly, the cerebral cortex was isolated and placed into Hanks' balanced salt solution (HBSS) containing 0.125% trypsin. After removal of the meninges, the tissue was transferred to 0.06% trypsin and digested for 30 min at room temperature, followed by mechanical trituration with pipettes. The number of viable cells was counted with the trypan blue exclusion method. Subsequently, cells were plated on glass coverslips placed in 6-well plates coated with poly-L-ornithine at a density of 6.7×10^5 cells/well. The neurons were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% heat-inactivated fetal bovine serum and F12 with penicillin (100 IU/ml) and streptomycin (100 μ g/ml) at 37°C in a humidified atmosphere of 95% air – 5% CO₂. Two days after plating, non-neuronal cells were inhibited by the addition of cytosine arabinoside (10 μ M), and the next day, the culture media were changed to NeuroBasal supplemented with B27, penicillin (100 IU/ml) and streptomycin (100 μ g/ml). Experiments were carried out in 10- to 14-day-old cultures.

MeHg treatment

MeHg was dissolved in HEPES Buffer (pH 7.4) to create a 1 mM stock solution. From this stock solution, appropriate work dilutions were made. After two washes with HEPES buffer, astrocytes or neurons grown in solitary conditions were treated with 10 μ M or 1 μ M MeHg in HEPES buffer (in the absence of serum) for 6 hrs. To examine the interaction between

astrocytes and neurons, neurons cultured on glass coverslips were carefully removed and placed in direct contact over astrocytes growing in the well of 6-well culture plates. The treatment procedures were identical in solitary astrocyte and neuron cultures, as well as in the co-culturing model. At the end of the experiments, the culture medium was aspirated from the neuronal or astrocytic cultures, and the cells were washed three times with HEPES buffer and then scraped into iced $1 \times$ PBS. For the co-cultured cells, the glass coverslips with neurons were carefully removed from the astrocytes and placed into new 6-well plates. Astrocytes and neurons were collected separately to allow for amino acid analysis. The cell pellets were solicited and centrifuged at $16,000 \times g$ to remove cellular debris. Protein was determined by the bicinchoninic acid (BCA) assay (Pierce, Rockford, IL, USA) to allow for standardization across the different cell cultures.

Measurements of Amino Acids

Samples were analyzed with a Hitachi L-8800 High Performance Amino Acid Analyzer. The analyzer uses a cation-exchange column with a gradient pH and ionic strength buffer elution followed by post-column derivation with ninhydrin. Areas of the internal standard were compared with each chromatograph to evaluate the precision of each injection. The retention times and peak areas were compared to an amino acid standard in order to calculate the concentrations of each amino acid. Sulfosalicylic acid (Sigma catalog# S-2130) was used as an internal standard because its retention time is sufficiently removed from other amino acids of interest. Basic amino acid standards (sigma catalog # A6282) and acidic amino acid standards (sigma catalog# A6407) were combined and used as standards. The contents of amino acids of interest were calculated as nmols per μg protein.

Statistical Analysis

Results were analyzed with the SPSS system v11.5 statistical analysis package (SPSS Inc., Chicago, IL, USA). Two-way analysis of variance (ANOVA) with between-groups factors (MeHg and cell type) to test for interactions between MeHg treatment and cell culture method was used. Tukey's HSD post-hoc test was used to evaluate mean differences. The alpha level for the analyses was set at $p < 0.05$.

Results

As shown in Figure 1, glutamate levels in neuronal cultures exposed to $10 \mu M$ MeHg were significantly decreased ($p < 0.05$ vs. control); however, this effect was fully reversed in neurons co-cultured in the presence of astrocytes.

Similarly, as shown in Figure 2, serine concentrations decreased by 20% and 27% in astrocytes treated with 1 or $10 \mu M$ MeHg ($p < 0.05$ vs. control), respectively, and 32% in neurons exposed to $10 \mu M$ MeHg ($p < 0.05$ vs. control). However, when treatments were carried out in the co-culture model, MeHg had no effect on serine levels in either of the cell types, and the levels of serine were indistinguishable from controls.

As shown in Figure 3, upon treatment with $10 \mu M$ MeHg, glycine concentrations were significantly decreased ($p < 0.05$ vs. control) both in astrocytes and neurons cultured in solitary conditions. In co-cultured astrocytes and neurons, the effect of MeHg was fully reversed, and levels of glycine in either of the cell types were indistinguishable from their respective controls.

As shown in Figure 4, the concentrations of alanine were also significantly ($p < 0.05$ vs. control) decreased in solitary astrocyte or neuron cell cultures upon treatment with $10 \mu M$ MeHg. In the co-culture model, the effect of MeHg was fully reversed in the astrocytes, such that alanine levels were indistinguishable from controls. Furthermore, the effect of $10 \mu M$ MeHg on

neuronal alanine concentrations was fully reversed under co-culturing conditions, leading to a significant increase ($p < 0.05$) in alanine levels.

As shown in Figure 5, the aspartate concentration was significantly decreased ($p < 0.05$ vs. control) in solitary neurons exposed to 10 μM MeHg. This effect of MeHg, however, was fully reversed in the co-culture model, such that aspartate content in co-cultured neurons was indistinguishable from the controls.

Discussion

The present study aimed to compare the effects of the exposure of cultured astrocytes and neurons to MeHg on the contents of a number of amino acids that are critical for carrying out and/or metabolically regulating amino acid neurotransmission. We decided to extend the examination of cells grown in solitary conditions to a co-culture system as we posited that the interaction between the two cell types would lessen the MeHg-induced changes in amino acid levels inherent to neurons, and perhaps also in astrocytes cultured in solitary conditions. The results obtained largely confirmed this hypothesis: MeHg-induced decreases in the content of several amino acids in solitarily culturing conditions were fully attenuated in the co-culture model.

While several studies have shown that MeHg affects glutamate homeostasis in astrocytes (Aschner et al., 1990; 2000; Farina et al., 2003; Qu et al., 2003) or neurons (Allen et al., 2001b; Allen et al., 2002; Morken et al., 2005), the interactive responses of the two cell types have yet to be fully explored. Furthermore, no studies have investigated the effects of MeHg on other amino acids, such as alanine, glycine and serine. To our knowledge, this is the first study to directly assess the effect of MeHg on amino acid levels in cerebral cortical neurons and astrocytes cultured in isolation, and to determine whether these effects may be modulated and reversed by the co-culturing conditions. Results from the present study established that the contents of the amino acids, glutamate, aspartate, alanine, glycine and serine significantly decreased in neuronal cultures exposed to 10 μM MeHg, and much less so – if at all – in astrocytes. Amino acids were maintained at levels indistinguishable from controls in the co-culture model, both in the presence of 1 or 10 μM of MeHg. The exception was alanine, as its levels increased in co-cultured neurons exposed to the high concentration of MeHg. These results indicate that astrocytes are not only more resistant to MeHg than neurons, but that, when cultured together with neurons, they can mitigate the MeHg-induced effects normally observed in neurons cultured in the absence of astrocytes. In addition, changes in astrocytes in co-culture were less pronounced than in astrocytes cultured alone, indicating that shielding by the interaction between the two cell types may be mutual.

The present study showed that, in response to MeHg treatments, the contents of aspartate and glutamate in neuronal cultures significantly decreased compared with relative controls. In contrast, the contents of aspartate and glutamate in astrocytes were indistinguishable from controls (Figures 1, 5). These results are consistent with other reports that neurons are significantly more sensitive to MeHg exposure than astrocytes. For example, MeHg was previously shown to exert a greater effect on glutamate metabolism in cortical neurons vs. astrocytes (Allen et al., 2001b; Allen et al., 2002; Morken et al., 2005). While the nature of the effect has yet to be fully defined, it is likely that neuronal MeHg exposure leads to disturbances in mitochondrial function (Allen et al., 2001c; Qu et al., 2003), perturbing mitochondrial permeability transition and, thereby, leading to the collapse of the mitochondrial inner membrane potential ($\Delta\Psi_m$) (Yin et al., 2007). Mitochondrial membrane damage, in turn, likely leads to the dysfunction of the malate-aspartate shuttle, thus impairing the influx of malate and glutamate and the efflux of aspartate and α -ketoglutarate from the mitochondria, which may account for the noted changes in neuronal aspartate and glutamate concentrations upon

exposure to MeHg (Fig. 6). The malate-aspartate shuttle represents a major pathway for the influx of malate and glutamate, as well as the efflux of aspartate and α -ketoglutarate from the mitochondria for oxidative metabolism (McKenna et al., 2006). It is particularly important in neurons (Fitzpatrick et al., 1983; Cheeseman and Clark., 1988; Lai et al., 1989) and its activity is extremely low and largely undetectable in astrocytes (Ramos et al., 2003). These effects of MeHg are not as pronounced in solitary astrocyte cultures, which is most likely due to their higher GSH content as compared to neurons (Yudkoff et al., 1990; Aschner et al., 1994). Studies demonstrate that MeHg neurotoxicity is associated with the production of reactive oxygen species (ROS) both *in vitro* (Ali et al., 1992; Sorg et al., 1998; Shanker et al., 2005; Kaur et al., 2006) and *in vivo* (Farina et al., 2005; Bellum et al., 2007). GSH plays a key role in detoxifying ROS (Jain et al., 1991; Dringen et al., 1999; Kaur et al., 2006) as the major antioxidant in mammalian cells (Anderson and Meister 1983). Thus, despite the preferential accumulation of MeHg in astrocytes (Garman et al., 1975; Barbour et al., 1988; Charleston et al., 1996; Aschner et al., 2002), they may be more resistant to the neurotoxic effects of this metal.

Our results also indicate that the microenvironment in which the cells are cultured changes the vulnerability of neurons and astrocytes to MeHg. This notion is consistent with earlier observations that neuronal vulnerability to glutamate toxicity is 40– to 100–fold higher in astrocyte-poor cultures than in astrocyte-rich cultures (Rosenberg and Aizenman 1989), and that glutamate uptake is 1/25 as active in astrocyte-poor cultures as in astrocyte-rich cultures (Rosenberg et al., 1992). In astrocyte-poor cultures, both glutamate and NMDA have access to dendrites, the most sensitive target for glutamate toxicity because of the location of excitatory synapses on these structures (Harris and Rosenberg 1993). In addition, the difference in neuronal vulnerability may be due to the alteration of the access of glutamate to neurons because of shielding by astrocytes and the powerful astrocyte uptake system (Barbour et al., 1988; Connors et al., 1988). These factors may account for the observation that the effects of MeHg on glutamate and aspartate in neurons are different in solitary cultures from those in co-culture conditions.

The intracellular concentration of alanine was significantly decreased upon treatment with 10 μ M MeHg in solitarily cultured neurons and astrocytes. However, under co-culturing conditions, alanine content in both cell types remained at levels indistinguishable from controls (Figure 4). Alanine and lactate constitute an intercellular shuttle, which transfers ammonia (NH_4^+) for glutamine/glutamate synthesis (Figure 6). Given the marked reduction in neuronal glutamate concentration upon exposure to MeHg, it is likely that decreased alanine concentrations reflect the interruption of the lactate-alanine shuttle by the reduced levels of glutamate, or alternatively, MeHg may directly inhibit the activity of alanine aminotransferase (ALAT), effectively reducing neuronal concentrations of glutamate and alanine (Figure 6). The last hypothesis is likely because ALAT has sulfhydryl groups that are crucial for its catalytic activity (Matsuzawa and Segal, 1968) and that might be oxidized by MeHg. The re-establishment of alanine levels in neurons under the co-culture condition might be consequence of the contribution of lactate supplied by astrocytes. We also looked at neuronal tyrosine and other amino acids, such as leucine, arginine, histidine and phenylalanine, and we found that MeHg had no effect on the neuronal content of these amino acids, with their levels corresponding to controls (data not shown). This is noteworthy, as it suggests that the effects of MeHg on amino acid content are selective.

Additional studies were directed at the consequences of MeHg exposure on L-serine and glycine homeostasis. L-serine is utilized for the synthesis of a variety of molecules, including glycine (Mitoma et al., 1998; Furuya et al., 2000). It is well established that the addition of an astrocyte-conditioned medium to cultured neurons dramatically improves neuronal survival, as well as axonal and dendritic growth (Banker 1980). Recently, it was noted that L-serine in

an astrocyte-conditioned medium serves as a major active trophic molecule and a potent survival-promoting factor for neurons (Mitoma et al., 1998; Furuya et al., 2000). Adult neurons lose the ability to synthesize L-serine and thus depend on astrocytes for their supply of this amino acid (Yamasaki et al., 2001).

Analogous to the noted decrease in neuronal glutamate content upon MeHg treatment (Figure 1), we observed a significant decrease in neuronal L-serine and glycine levels (Figures 2 and 3, respectively). When co-cultured with astrocytes, neuronal serine and glycine levels increased but were indistinguishable from those noted in control neurons, paralleling the changes in glutamate levels. However, in astrocytes, L-serine levels significantly decreased in cells exposed to low and high MeHg concentrations (Figure 2), even though glutamate levels remained normal (Figure 1). In the presence of MeHg, cysteine combines with MeHg (Carty and Malone 1979); MeHg also inhibits cysteine and cystine uptake in astrocytes, but not in neurons (Allen et al., 2001a; Shanker et al., 2001). Under such conditions, more cysteine, a precursor of GSH, is utilized to maintain GSH levels, further decreasing the content of cysteine. As a precursor of cysteine, the serine concentration consequently decreases. This result may represent a mechanism accounting for the decrease in serine concentrations in astrocytes exposed to MeHg.

In summary, the present study shows, for the first time, that in addition to its previously documented effects on glutamate homeostasis, MeHg also alters the astrocytic concentrations of several other amino acids (serine, glycine, alanine). The content of these amino acids in cortical neurons is also altered in the presence of MeHg. The levels of these amino acids in both co-cultured neurons and astrocytes were indistinguishable from controls. This may be accounted for by the “buffering” of MeHg by astrocytes in the co-culture system, effectively shielding and protecting the neurons. On the other hand, the fact that amino acid content in astrocytes was likewise less affected by MeHg in the co-culture paradigm indicates that cross-talk between neurons and astrocytes in co-culture, possibly including the shuttling of amino acids, may elicit some kind of glioprotective function by neurons.

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Abbreviation

α-KG	α -ketoglutarate
ace CoA	acetyl coenzyme A
Ala	alanine
ALAT	alanine aminotransferase
cyt	cytosolic
GS	glutamine synthetase
Lac	

	lactate
LDH	lactate dehydrogenase
mGDH	mitochondrial glutamate dehydrogenase
PAG	phosphate-activated glutaminase
Pyr	pyruvate
TCA	tricarboxylic acid
CNS	central nervous system
GS	glutamine synthetase
GSH	glutathione
MeHg	methylmercury

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Figure 1.

Glutamate concentrations expressed as percent of control (no MeHg treatment). Glutamate concentrations in solitary astrocytic cultures or co-cultured (cc) with neurons were indistinguishable from controls in the presence of MeHg in both low and high concentrations; however, glutamate concentrations in neurons cultured alone significantly decreased upon treatment with 10 μ M MeHg. When neurons were cultured with astrocytes (cc), the effect of MeHg on glutamate concentrations was fully attenuated. Effect of the culture type on glutamate concentrations $F_{(3,11)}=18.586$, $p<0.001$; effect of treatment on glutamate concentrations: $F_{(2,11)}=1.198$, $p=0.149$; interaction between culture type and treatment: $F_{(6,11)}=4.348$, $p<0.001$. Values represent mean $\pm$ SEM of three to five independent experiments. Values are corrected for protein concentrations. * $p<0.05$ vs. control.

Figure 2.

Serine concentrations expressed as percent of control. Serine concentrations significantly decreased in astrocytes and neurons cultured in solitary conditions in the presence of 10 μ M MeHg. Serine concentration also significantly decreased in astrocytes in solitary conditions exposed to 1 μ M MeHg. These effects were reversed in co-cultured astrocytes and neurons exposed to 1 or 10 μ M MeHg, respectively. Effect of culture type on serine concentrations: $F(3,11)=19.319$, $p<0.001$; effect of treatment on serine concentrations: $F(2,11)=2.433$, $p=0.089$; interaction between culture type and treatment: $F(6,11)=1.741$, $p=0.111$. Values represent mean $\pm$ SEM of three to five independent experiments. Values are corrected for protein concentrations. * $p<0.05$ vs. control.

Figure 3.

Glycine concentrations expressed as percent of control. Glycine concentrations significantly decreased in astrocytes and neurons cultured in solitary conditions in the presence of 10 μ M MeHg. These effects were reversed in co-culture conditions. Effect of culture type on glycine concentrations: $F_{(3,11)}=6.487$, $p<0.001$; effect of treatment on glycine concentrations: $F_{(2,11)}=10.387$, $p<0.001$; interaction between culture type and treatment: $F_{(6,11)}=1.690$, $p=0.123$. Values represent mean $\pm$ SEM of three to five independent experiments. Values were corrected for protein concentrations. * $p<0.05$ vs. control.

Figure 4.

Alanine concentrations expressed as percent of control. Alanine concentrations significantly decreased in astrocytes and neurons cultured in solitary conditions in the presence of 10 μ M MeHg. These effects were reversed in co-cultured astrocytes and neurons, except that, in co-cultured neurons, alanine concentrations significantly increased ($p < 0.05$ vs. control) upon treatment with 10 μ M MeHg. Effect of culture type on alanine concentrations: $F_{(3,11)} = 4.793$, $p = 0.003$; effect of treatment on alanine concentrations: $F_{(2,11)} = 2.670$, $p = 0.071$; interaction between culture type and treatment: $F_{(6,11)} = 0.858$, $p = 0.526$. Values represent mean $\pm$ SEM of three to five independent experiments. Values are corrected for protein concentrations. * $p < 0.05$ vs. control.

Figure 5.

Aspartate concentrations expressed as percent of control. Aspartate concentration decreased significantly in neurons cultured in solitary conditions upon treatment with MeHg at 10 μ M. This effect was reversed in co-culture conditions. The concentration of aspartate in astrocytes in solitary and co-culture conditions was indistinguishable from controls in the presence of MeHg. Effect of culture type on aspartate concentrations: $F_{(3,11)}=9.564$, $p<0.001$; effect of treatment on aspartate concentrations: $F_{(2,11)}=5.914$, $p=0.003$; interaction between culture type and treatment: $F_{(6,11)}=1.896$, $p=0.097$. Values represent mean $\pm$ SEM of three to five independent experiments. Values are corrected for protein concentrations. * $p<0.05$ vs. control.

Figure 6.

Schematic model of a glutamate-glutamine/lactate-alanine/malate-aspartate shuttle between neurons and astrocytes. Glucose is processed to lactate by astrocytes. Lactate is either metabolized via the TCA cycle or used for exchange between astrocytes and neurons, accompanied by exchange of alanine from neurons to astrocytes. In astrocytes, alanine provides NH_4^+ for glutamine synthesis from GDH and GS. Subsequently, glutamine released from astrocytes is taken-up by neurons for the re-synthesis of glutamate by PAG, which is preferentially expressed in neurons. Meanwhile, lactate is transferred into alanine via ALAT, with which MeHg may potentially react. Glutamate, which can have its uptake and efflux affected by MeHg, is released from neurons and taken up by astrocytes to synthesize glutamine using NH_4^+ generated from the conversion of glutamate to α -ketoglutarate by GDH. Glutamine, disturbed by the presence of MeHg, is released and taken up by neurons where the mitochondrial enzyme, PAG, forms NH_4^+ for the production of glutamate via GDH. Oxaloacetate (OAA) is converted to aspartate by transamination with glutamate via mitochondrial aspartate aminotransferase (mAAT). Aspartate uptake, which is inhibited by MeHg, exits the mitochondria via the aspartate-glutamate carrier in exchange for glutamate. In the cytosol, aspartate is converted to OAA by transamination with α -ketoglutarate via cytosolic aspartate aminotransferase (cAAT). OAA is subsequently converted to malate by cytosolic malate dehydrogenase (cMDH). Malate enters the mitochondrial matrix via the malate- α -ketoglutarate carrier in exchange for α -ketoglutarate. Malate is oxidized to OAA by mitochondrial malate dehydrogenase (mMDH), thus completing the shuttle. MeHg can react with acetyl-CoA and Suc-CoA to interrupt the TCA cycle; MeHg may react on the sites where the enzymes and carriers are located.