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Mechanisms of methylmercury-induced neurotoxicity: evidence from experimental studies

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Abstract

Neurological disorders are common, costly, and can cause enduring disability. Although mostly unknown, a few environmental toxicants are recognized causes of neurological disorders and subclinical brain dysfunction. One of the best known neurotoxins is methylmercury (MeHg), a ubiquitous environmental toxicant that leads to long-lasting neurological and developmental deficits in animals and humans. In the aquatic environment, MeHg is accumulated in fish, which represent a major source of human exposure. Although several episodes of MeHg poisoning have contributed to the understanding of the clinical symptoms and histological changes elicited by this neurotoxicant in humans, experimental studies have been pivotal in elucidating the molecular mechanisms that mediate MeHg-induced neurotoxicity. The objective of this mini-review is to summarize data from experimental studies on molecular mechanisms of MeHg-induced neurotoxicity. While the full picture has yet to be unmasked, *in vitro* approaches based on cultured cells, isolated mitochondria and tissue slices, as well as *in vivo* studies based mainly on the use of rodents, point to impairment in intracellular calcium homeostasis, alteration of glutamate homeostasis and oxidative stress as important events in MeHg-induced neurotoxicity. The potential relationship among these events is discussed, with particular emphasis on the neurotoxic cycle triggered by MeHg-induced excitotoxicity and oxidative stress. The particular sensitivity of the developing brain to MeHg toxicity, the critical role of selenoproteins and the potential protective role of selenocompounds are also discussed. These concepts provide the biochemical bases to the understanding of MeHg neurotoxicity, contributing to the discovery of endogenous and exogenous molecules that counteract such toxicity and provide efficacious means for ablating this vicious cycle.

Keywords

methylmercury; neurotoxicity; oxidative stress; glutamate; calcium; selenium; selenoproteins; glutathione peroxidase

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Introduction

Neurological disorders represent an important health concern faced by society, accounting for great numbers of hospitalizations and disabilities (Schmidt, 2007). Although still debatable, evidence has pointed to a potential link between exposures to chemical contaminants and neurological disorders/mental illness, such as autism (Landrigan, 2010), Parkinson's disease (Barlow et al., 2007), Alzheimer's disease (Coppede and Migliore, 2010), amyotrophic lateral sclerosis (Johnson and Atchison, 2009), just to name a few. Important chemical contaminants reported to cause neurotoxic effects in humans at environmentally relevant levels of exposure include polychlorinated biphenyls (Schantz et al., 2003), arsenic (Wasserman et al., 2004), lead (Lanphear et al., 2005), manganese (Takser et al., 2003), pesticides (Rauh et al., 2006), polycyclic aromatic hydrocarbons (Perera et al., 2006) and mercury compounds (Grandjean et al., 1997; Debes et al., 2006).

Mercury (Hg) is present in the environment due to either natural events or anthropogenic sources. It exists within the environment in three different chemical forms: elemental mercury vapor, inorganic mercury salts, and organic mercury (Clarkson, 1997). The distribution, toxicity and metabolism of mercury are greatly dependent on its chemical form. Organic mercury compounds, such as methylmercury (MeHg), have been extensively studied because they are able to reach high levels in the central nervous system (CNS), leading to neurotoxic effects (Aschner et al., 2007; Clarkson and Magos, 2006).

MeHg is an environmental contaminant that has been shown to cause neurological deficits in both animals and humans (Clarkson et al., 2003; Farina et al., 2010). As a result of the biomethylation of mercury compounds released mainly from anthropogenic sources in the aquatic environment, MeHg-containing fish represent a major source of human exposure. Therefore, fishing communities, which commonly depend greatly on fish for food, can be exposed to toxic levels of MeHg (Clarkson et al., 2003).

Human poisoning to MeHg was first reported around 150 years ago in laboratory accidents (Edwards, 1865). Several years later, catastrophic epidemics from environmental MeHg contamination in Japan (Harada, 1978) and Sweden (Westöö, 1966) were reported. Subsequently, exposure was documented in Iraq where locals consumed bread prepared from seeds treated with a fungicide containing MeHg (Bakir et al., 1973), causing a large outbreak of human poisoning. Similar incidents occurred in Guatemala, Pakistan, and Ghana (for a review, see Clarkson, 2002). Observed among the initial symptoms of MeHg poisoning were paresthesias, constriction of visual fields, impairment of hearing and speech, cerebellar ataxia and psychiatric symptomatology (Ekino et al., 2007; Murata et al., 2007).

Although the aforementioned episodes contributed to the understanding of the main clinical symptoms and histological changes elicited by MeHg poisoning in humans, the molecular mechanisms involved have been elucidated mainly based on studies with laboratory animals. Moreover, important aspects related to MeHg's absorption, distribution, storage, biotransformation and elimination were also clarified due to the development of experimental studies. This mini-review discusses the importance of experimental studies to the understanding of molecular mechanisms related to MeHg-induced neurotoxicity. The correlation between biochemical and behavioral effects in MeHg-exposed animals, the relationship between oxidative stress and glutamate dyshomeostasis, and the potential use of antioxidant selenocompounds to counteract MeHg-induced neurotoxicity in experimental models are the focus of this mini-review.

Behavioral hallmarks

Behavioral parameters detected in MeHg-exposed animals have been essential to the understanding of the bases of several clinical manifestations observed in humans. In the early 1950s, an epidemiological investigation at the Minamata district showed that several cats fed a diet that primarily consisted of fish presented a severe condition named “dancing disease”, which was characterized by movement disorders, convulsions and death (Kitamura et al., 1957). Another epidemiological report showed that fish-feeding cats that lived on Indian Reserves in Northwestern Ontario, Canada, developed acute neurological symptoms characterized by ataxic gait, abnormal movements, uncontrolled howling, and seizures (Takeuchi et al., 1977). The foregoing evidences (Kitamura et al., 1957; Takeuchi et al., 1977) pointed to motor impairment as an important behavioral sign observed in MeHg-poisoned animals, corroborating data in humans (Ekino et al., 2007; Murata et al., 2007). Such symptoms were also observed in *experimental studies* using cats (Charbonneau et al., 1976), dogs (Mattsson et al., 1981), mice (Inouye et al., 1985; Dietrich et al., 2004); rats (Rocha et al., 1993; Farina et al., 2005), monkeys (Rice, 1996) and zebrafish (*Danio rerio*) (Samson et al., 2001). Although experimental studies on MeHg-exposed animals have shown that behaviors related to visual (visual contrast sensitivity task; Burbacher et al., 2005), cognitive (passive avoidance task; Ferraro et al., 2009) and emotional (depression-like behavior evaluated in the forced swimming test; Onishchenko et al., 2007) functions are also affected by MeHg exposures, *movement disorders*, consisting mainly of ataxia and loss of balance, have been extensively reported in experimental studies using MeHg-exposed animals (Dietrich et al., 2004, Farina et al., 2005; Lucena et al., 2007) and have been used as an important behavioral parameter to correlate with histological/cellular damage and/or biochemical changes (Franco et al., 2006; Carvalho et al., 2007, Carvalho et al., 2008), as well as to study potential protective/antidotal strategies to counteract MeHg-induced toxicity (Farina et al., 2005; Martins et al., 2009).

As mentioned previously, fishing communities, which commonly present a fish-based diet, can be exposed to toxic levels of MeHg (Clarkson et al., 2003). Accordingly, oral exposure represents a major form by which human population is exposed to MeHg. It is noteworthy that MeHg absorption in the gastrointestinal (GI) tract is around 90–95%, which is significantly higher when compared to the GI absorption of inorganic salts of mercury from food (Nielsen, 1992). Taking these kinetic properties into consideration, and aiming to better understand the movement alterations elicited by MeHg exposure, our group standardized an exposure protocol based on the intoxication of Swiss mice through the ingestion of MeHg (Dietrich et al., 2004). In this protocol, methylmercury (II) chloride (CH_3HgCl) is diluted in drinking water and animals are allowed to drink the MeHg solution, *ad libitum*. We have observed that the liquid ingestion of MeHg-exposed mice is similar to that of control animals (allowed to drink tap water) when MeHg concentrations do not exceed 40 mg/L (please, consider that the use of different strains or species should give different effects). Using higher concentrations (e.g. 60 mg/L), mouse liquid ingestion is significantly decreased probably due to the metallic taste of MeHg which causes food aversion in the mice. Based on the daily liquid ingestion of an adult Swiss mouse (around 6.5 ml/day) and on its body weight (around 45 g), a daily MeHg dose of 7 mg/kg body weight can be calculated for animals exposed to a 40 mg/L MeHg solution, *ad libitum*. This dose is similar to those in toxicological studies based on either intraperitoneal or subcutaneous injections (Kobayashi et al., 1981; Verschaeve and Léonard, 1984; Stringari et al., 2006). The advantage of using an oral exposure protocol is its similarity with the most common human exposure condition, as well as the absence of daily injections and the associated discomfort. The protocol is also practical for relatively long-term exposure periods (several months if using low doses). From an environmental point of view, this is important because long-term

exposures to MeHg in experimental studies better represent the chronic exposure in humans, who are normally exposed to the neurotoxicant for months or years.

As already mentioned, motor alterations represent an important behavioral outcome of MeHg poisoning in both animals (Charbonneau et al., 1976; Mattsson et al., 1981; Inouye et al., 1985; Dietrich et al., 2004; Rocha et al., 1993; Farina et al., 2005; Rice, 1996) and humans (Ekino et al., 2007; Murata et al., 2007). The aforementioned experimental protocol (Dietrich et al., 2004) is able to induce significant deficits on motor performance in Swiss mice, which can be observed with different low-cost tests, such as the footprint (Carter et al., 1999), beam walking (Perry et al., 1995) and rotarod (Jones and Roberts, 1968) tests. Figure 1 depicts the progressive loss of the motor coordination (normal gait) in animals exposed to MeHg in drinking water.

Gender is an important variable to take into consideration when studying MeHg-exposed animals and evaluating motor impairments. An interesting finding obtained from mice exposed to MeHg via drinking water is that female mice are more resistant to MeHg-induced motor impairment compared to males (Malagutti et al., 2009). Consistent with this observation, when studied in the rotarod apparatus, two-week exposure to MeHg in drinking water (40 mg/L, *ad libitum*) caused a significant motor impairment in male but not female adult Swiss mice (Malagutti et al., 2009). Data from other experimental studies (Gimenez-Llort et al., 2001) have also pointed to more profound neurotoxic effects (spontaneous and dopamine-stimulated locomotor activity) of MeHg in males, corroborating epidemiological studies in humans exposed to MeHg (McKeown-Eyssen et al., 1983; Grandjean et al., 1998). Since the co-administration of 17 β-estradiol protected male mice against the neurotoxic effects induced by oral MeHg exposure (Malagutti et al., 2009), it is reasonable to suggest that sex steroids afford neuroprotective effects.

Another important characteristic of the proposed protocol (exposure of Swiss mice to MeHg through the ingestion of contaminated water) is that the mercury levels in central structures of these mice closely mimic those in brains obtained from autopsied humans (Myers et al., 1995; Franco et al., 2006), reflecting upon the environmental significance of the exposure protocol.

Molecular mechanisms of neurotoxicity in experimental animal models

As mentioned above, experimental studies in either *in vitro* or *in vivo* models have been crucial in elucidating the molecular and cellular mechanisms of neurotoxicity elicited by MeHg. Pioneering *in vivo* studies with ^{203}Hg showed that the brain uptake of MeHg in rats is enhanced by continuous L-cysteine infusion (Aschner and Clarkson, 1987). This study brought about the hypothesis that MeHg may be transported from the blood to the CNS across the blood-brain barrier (BBB) by the L-type neutral amino acid carrier transport (LAT) system. A few years later, Kerper and collaborators (1992) observed that MeHg entered the rat brain as a cysteine complex via the LAT system as a result of the close mimicry between the MeHg/L-cysteine (MeHg-Cys) complex and L-methionine, a substrate for the LAT amino acid transport system. More recently, an *in vitro* experimental study using cultured CHO-k1 (Chinese hamster ovary) cells showed that the overexpression of the L-type large neutral amino acid transporter, LAT1, was associated with increased uptake of MeHg in the presence of L-cysteine, as well as reduced cellular viability (Yin et al., 2008). These observations culminated in the conclusion that MeHg-L-cysteine conjugate (MeHg-Cys) is a substrate for the LAT1 system, which actively transports MeHg across membranes and is responsible, at least in part, for the high Hg levels found in the brain after exposures. Indeed, although other transporters have been reported to contribute to MeHg transport (Whu, 1996; Bridges and Zalups, 2010), LAT1 seems to be the main, if not the only,

transporter responsible for MeHg transport from peripheral tissues to the CNS (Kerper et al., 1992). Interestingly, a recent *in vitro* study in tissue slices showed that methionine pre-treatment displayed a protective effect against the toxic effects induced by MeHg and/or MeHg-Cys on mitochondrial function and cell viability, suggesting that methionine can be considered a potential strategy to the treatment of acute MeHg exposure (Roos et al., 2011).

Not only the transport and metabolism of MeHg, but also its major molecular targets and biochemical effects have been elucidated in experimental studies. Of particular importance, *in vivo* studies with rats showed that MeHg combines covalently with sulfhydryl (thiol) groups from plasma cholinesterase, leading to the enzyme inhibition (Hastings et al., 1975). After this important observation, several *in vitro* and *in vivo* experimental studies showed that sulfhydryl-containing enzymes are inhibited by MeHg (Kanda et al., 1976; Magour et al., 1986; Kageyama et al., 1986; Rocha et al., 1993; Kung et al., 1987). These observations led to the notion that the direct chemical interaction among MeHg and thiol groups from proteins and non-protein molecules, such as glutathione (GSH; γ -glutamyl-cysteinyl-glycine), plays a crucial role in MeHg-induced neurotoxicity (for a review, see Aschner and Syversen, 2005).

The antioxidant GSH system is an important target in mediating MeHg neurotoxicity (Kaur et al., 2006; Stringari et al., 2008). GSH is the most abundant intracellular low molecular weight thiol compound in all tissues, including the CNS (Dringen, 2000). GSH is present at the low millimolar range (1–10 mM) in some mammalian cells (Cooper and Kristal, 1997). Its reducing capacity is determined by the nucleophilic properties of its thiol group and its antioxidant role is sustained by the presence of several enzymes that catalyze its interaction with endogenous and xenobiotic electrophilic molecules (Zhu et al., 2006). Of particular importance, glutathione peroxidase (GPx) and glutathione reductase (GR) are central enzymes involved with detoxification of peroxides and reduction of glutathione disulphide (oxidized glutathione; GSSG), respectively (Dringen, 2000). The precise activities of these enzymes, as well as the maintenance of a normal thiol status, represented mainly by the GSH/GSSG ratio, are crucial for protecting cells against oxidative damage. With particular emphasis on the toxicity induced by MeHg, it is known that its mercury atom directly interacts with the thiol group of GSH, leading to the formation of an excretable GS-$HgCH_3$ complex (Ballatori and Clarkson, 1982). This interaction has been proposed to decrease the levels of GSH, which could contribute to the occurrence of oxidative stress (Franco et al., 2007). Consistent with this hypothesis, experimental studies based on *in vitro* approaches have reported decreased GSH levels after MeHg exposure. This effect was detected in non-neuronal cell lines (Amonpatumrat, 2008), neuronal and glial primary cultures (Kaur et al., 2006) and isolated mitochondria from the mouse brain (Franco et al., 2007). From a molecular point of view, decreased GSH levels, which can occur as a consequence of GS-$HgCH_3$ complex formation, will cause increased reactive species (H_2O_2, nitric oxide, etc.) generation and oxidative damage in a plethora of biomolecules (nucleic acids, lipids and proteins). However, it is important to note that MeHg can induce oxidative stress due to its direct interaction with nucleophilic protein groups (Farina et al., 2010), even in the absence of significant changes in GSH levels or GSH/GSSG ratio.

Experimental *in vivo* studies have also reported decreased GSH levels in the CNS (cerebellum) after MeHg exposure in mice, although this effect was dependent upon ontogeny: weanling animals were more susceptible than adults (Franco et al., 2006). Another interesting *in vivo* study showed that prenatal MeHg exposure hampered the normal maturation of the antioxidant GSH system during the early postnatal period (Stringari et al., 2008). In fact, *in utero* exposure to MeHg, which did not alter cerebral GSH levels and GR activity at birth, inhibited the developmental profile of the cerebral GSH antioxidant system during the early postnatal period. Decreased GSH levels and reduced GR activity were

observed in the brain of MeHg-exposed animals at weaning (postnatal day 21). Additional studies are required to affirm if this event is permanent or if levels of GSH and GR activity are normalized as the animals reach adulthood. Nevertheless, this effect is consistent with additional molecular mechanism by which MeHg affects the GSH antioxidant system in the developing CNS, thereby rendering the brain more susceptible to oxidants. MeHg's effects on GSH homeostasis are depicted in Figure 2.

Glutamate dyshomeostasis in the CNS represents another critical target in MeHg-induced neurotoxicity (for a review, see Aschner et al., 2007). Glutamate is the major excitatory neurotransmitter in the mammalian CNS, where it plays key roles in development, learning, memory and response to injury (Featherstone, 2010). However, glutamate at high concentrations at the synaptic cleft acts as a toxin, inducing neuronal injury and death (Meldrum, 2000; Ozawa et al., 1998). Glutamate-mediated neurotoxicity has been dubbed as "excitotoxicity", referring to the consequence of the overactivation of the *N*-methyl D-aspartate (NMDA)–type glutamate receptors, leading to increased Na^+ and Ca^{2+} influx into neurons (Choi, 1992; Pivovarova & Andrews, 2010). Increased intracellular Ca^{2+} levels are associated with the generation of oxidative stress and neurotoxicity (Lafon-Cazal, 1993; Ceccatelli et al., 2010). Accordingly, the control of extracellular levels of glutamate dictates its physiological/pathological actions and this equilibrium is maintained primarily by the action of glutamate transporters (such as GLAST, GLT1, and EAAC1) located on astrocytic cell membranes, which remove the excitatory neurotransmitter from the synaptic cleft, keeping its extracellular concentrations below toxic levels (Anderson and Swanson, 2000; Maragakis and Rothstein, 2001; Szydlowska & Tymianski, 2010).

Advances in understanding the role of glutamate dyshomeostasis in MeHg-induced neurotoxicity was derived from *in vitro* studies with cultured cells (neurons and astrocytes), as well as tissue slices, isolated synaptosomes and synaptic vesicles. Experimental *in vitro* approaches have shown that MeHg readily inhibits glutamate uptake into cultured astrocytes (Brookes and Kristt, 1989; Aschner et al., 2000). MeHg also inhibits the uptake of glutamate into rat synaptic vesicles (Porciúncula et al., 2003) and cerebral cortical slices (Moretto et al., 2005a), suggesting that increased glutamate levels in the extracellular milieu could represent a biochemical consequence of MeHg exposure. In agreement, experimental *ex vivo* studies showed decreased glutamate uptake into cerebral cortex slices in both adult (Farina et al., 2003a) and weanling (Manfroi et al., 2004) mice exposed to MeHg. MeHg also increases the spontaneous release of glutamate from mouse cerebellar slices (Reynolds and Racz, 1987) and cultured neuronal cells (Vendrell et al., 2007), suggesting that increased glutamate release also contributes to elevated extracellular glutamate levels after MeHg exposure. The molecular mechanisms mediating increased glutamate release and decreased glutamate uptake in MeHg toxicity are not yet completely understood. However, evidence shows that hydrogen peroxide, whose levels are increased during MeHg exposure, can be a crucial molecule involved in the oxidative and inhibitory effects on astrocyte glutamate transporters (Allen et al., 2001). On the other hand, increased glutamate release seems to depend, at least in part, on the decreased vesicular uptake of glutamate, probably due to the direct inhibition of H^+-ATPase activity (Porciúncula et al., 2003). Interestingly, the foregoing experimental *in vitro* evidences were confirmed by *in vivo* studies with microdialysis probes implanted in the frontal cortex of adult Wistar rats (Juarez et al., 2002), showing increased levels of extracellular glutamate after MeHg exposure.

All the aforementioned observations are consistent with the ability of MeHg to increase extracellular glutamate levels. The overactivation of NMDA-type glutamate receptors increases Ca^{2+} influx into neurons, therefore leading to the activation of important pathways involved with cell death. Alternatively, Ca^{2+} can be taken up by mitochondria, where it may stimulate the generation of reactive oxygen species (ROS) (Reynolds and Hastings, 1995).

Consistent with this hypothesis, several studies corroborates MeHg's ability to induce mitochondrial dysfunction and consequent generation of hydrogen peroxide (Mori et al., 2007), which by itself is a potent inhibitor of astrocytic glutamate uptake (Allen et al., 2001). Thus, three important events related to MeHg-induced neurotoxicity [(i) Ca^{2+} dyshomeostasis, (ii) glutamate dyshomeostasis, and (iii) increased ROS generation/oxidative stress] represent interrelated events that can feed forward upon each other. This connection between the aforementioned events is depicted in Figure 3.

Neurodevelopmental toxicity in experimental models

Experimental and epidemiological evidence indicates that compared to the adult CNS the developing CNS is more susceptible to several toxicants (Costa et al., 2004; Grandjean and Landrigan, 2006). With particular emphasis on the developmental neurotoxicity caused by MeHg, significant attention has been directed to this topic due to the long-term consequences of prenatal exposure to this organometal on child development in communities with chronic low level dietary exposure (Castoldi et al., 2008; Debes et al., 2006). In order to better understand the neurotoxicity induced by MeHg-exposure during the developmental period, different experimental protocols have been developed. At the beginning of the 1970s, Mansour and collaborators (1973) showed that MeHg is transferred across the placenta from pregnant rats to their fetuses. Few years later, a study on the pharmacokinetics of MeHg in the maternal-fetal unit in mice showed that the fetal accumulation of mercury increased with fetal age and peak fetal mercury was reached 3 days post MeHg administration (Oslon and Massaro, 1977), suggesting that the transfer of MeHg from pregnant mice to the fetus can occur within a relative short period (few days). Another interesting experimental study with pregnant mice exposed to MeHg showed significant changes in biochemical parameters in the fetuses' brain and absent changes in the mothers' brain (Watanabe et al., 1999). The results from Watanabe's work clearly showed that the transfer of MeHg from the pregnant animals to their fetuses via the placenta may lead to neurotoxicity in the offspring with no evident neurotoxic signs in their respective dams. Interestingly, epidemiological studies corroborated this observation in humans as well (Myers and Davidson, 2000), reinforcing the idea that the developing CNS is most susceptible to the deleterious effects of MeHg.

Epidemiological evidence indicates that prenatal MeHg exposure in humans may cause permanent neurological deficits (Debes et al., 2006). Experimental studies on the prenatal effects of MeHg showed a significant disruption in the postnatal development of the GSH antioxidant system (Stringari et al., 2008). Moreover, these authors observed significant delayed and long-lasting neurochemical changes during the suckling period following gestational MeHg exposure, even in the presence of extremely low levels of mercury in the brain (near to control values). These observations are consistent with the ability of exposure to MeHg during critical periods of development to trigger enduring and long-lasting (into adulthood) biochemical changes associated with oxidative stress (Debes et al., 2006).

Although astrocytes are the preferential site of MeHg accumulation (Charleston et al., 1996; Aschner, 1996), neurons seem to be more susceptible to MeHg-induced toxicity. With a particular emphasis on the developing brain, neuronal cells from different brain structures, such as hippocampus, cerebellum and cerebral cortex, have been reported as potential targets for the toxic effects elicited by MeHg (Burke et al., 2006; Gao et al., 2006; Falluel-Morel et al., 2007; Dasari & Yuan, 2009). With respect to the histological changes observed after MeHg exposure, significant reductions of the number of neurons have been particularly described (Carvalho et al., 2008; Falluel-Morel et al., 2007).

Although brain development is more dramatic during fetal life, it continues for years postnatally and additional exposure can occur when a mother breast-feeds or the child consumes fish (Myers et al., 2009). Thus, experimental studies on the developmental neurotoxicity elicited by postnatal MeHg-exposures appear to be also of toxicological significance. In this context, an experimental study on the lactational exposure and neonatal kinetics of MeHg in mice showed that postnatal exposure to MeHg via breast-feeding raises important toxicological concerns because of the high absorption of MeHg in the gastrointestinal tract, as well as its low excretion rate in pups (Sundberg et al., 1999). Taking into account that no neurotoxic signs were evaluated in Sundberg's work (1999), we conducted two experimental studies aimed at understanding the exclusive contribution of the lactational exposure to MeHg to neurotoxicity in the offspring (Manfroi et al., 2004; Franco et al., 2006). Female mice (genitors) were orally exposed to MeHg from the first day after parturition until postnatal day 21 (weaning period). Subsequently, weanling pups were exposed to MeHg exclusively by breast-feeding. The eventual pups' consumption of MeHg-containing water was excluded because the dispensing ends of the water tubes were not accessible to pups even at postnatal day 21. The major results from these two studies were that lactational exposure to MeHg induced several behavioral changes (mainly related to the motor function), as well as decreased glutamate uptake into cerebellar slices and increased cerebellar oxidative stress (Manfroi et al., 2004; Franco et al., 2006). These studies showed that the exclusive lactational exposure to MeHg caused neurotoxicity in the offspring and reinforced the link between glutamate dyshomeostasis, oxidative stress and movement deficits, which was previously observed in adult animals (Farina et al., 2003a; Farina et al., 2005). Although the foregoing studies on the lactational exposure to MeHg suggest that the presence of this toxicant in the breast milk can lead to neurotoxicity in experimental protocols with rodents, further epidemiological investigations are necessary to examine the occurrence of such events in humans.

MeHg interacts with selenol groups

As mentioned above, MeHg interacts with thiol groups from proteins and non-protein molecules, and such interaction plays a crucial role in MeHg-induced oxidative stress and neurotoxicity (Aschner and Syversen, 2005). Nevertheless, it is difficult to understand how the equimolar interaction between MeHg and GSH (the major low molecular weight thiol antioxidant) could lead to oxidative stress. In fact, GSH is present in some mammalian cells at concentrations near to 10 millimolar (Cooper and Kristal, 1997) and MeHg has been reported to cause oxidative damage in cultured neuronal cells when added to the culture medium at concentrations as low as 300 nanomolar (Farina et al., 2009). Moreover, *in vivo* experimental studies have shown reduced cerebellar (Franco et al., 2006) and cerebral (Stringari et al., 2008) GSH levels in MeHg-exposed animals whose cerebellar/cerebral mercury levels were in the low micromolar range. Taking into account these observations, it is reasonable to assume that nucleophilic groups other than GSH's thiol could be more reactive toward MeHg, allowing for the formation of high affinity complexes, thus leading to dysfunction of important cellular molecules with nucleophilic properties. In agreement with this hypothesis, thiol groups from specific proteins [tyrosine phosphatase (Sumi, 2008) and creatine kinase (Wang et al., 2001)] are more nucleophilic and, consequently, more reactive than GSH (Farina et al., 2010). Toxicological consequences of this phenomenon have already been reported (Glasser et al., 2010). Nonetheless, mammalian cells posses another group of proteins, named *selenoproteins*, whose nucleophilicity is in general higher than sulfhydryl-containing proteins. Selenoproteins are usually involved in redox reactions, where selenocysteine (a strong nucleophilic amino acid) is an active-site residue essential for catalytic activity (Lu and Holmgren, 2009). Selenocysteine possesses a selenol group (-SeH) that is generally more reactive than thiols (-SH), including towards mercury (Sugiura et al., 1976). Experimental studies have been pursued in order to understand the chemical

interaction between MeHg and selenol groups in the biological environment in an attempt to elucidate selenol's role in MeHg-induced neurotoxicity. In 1977, an *in vivo* experimental study with rats showed that the cerebral activity of glutathione peroxidase, a selenoprotein responsible for peroxide detoxification, was significantly decreased after MeHg exposure (Prohaska and Ganther, 1977). Interestingly, this effect was prevented by the equimolar administration of selenite. After this pioneering work, several studies showed that the selenium-mercury interaction presents important toxicological significance (for a review, see Khan and Wang, 2009). Recently, an experimental *in vitro study* with primary cultures of cerebellar neurons showed that glutathione peroxidase (isoform 1; GPx1) is an initial molecular target of low-dose MeHg (Farina et al., 2009). In fact, GPx1 activity was decreased in MeHg-treated neurons with no changes in several biochemical parameters that are normally affected by high-dose MeHg exposures. In addition, the decreased enzyme activity was a consequence of direct inhibitory effects, which were probably related to mercury-selenol interactions. Interestingly, these events were responsible for enhanced susceptibility to peroxides, increased lipid peroxidation and neuronal death (Farina et al., 2009). In agreement, another recent study established that glutathione peroxidase also serves as a crucial molecule involved with MeHg-induced neurotoxicity (Franco et al., 2009). In addition to the direct interaction between MeHg and the selenol group of GPx, another molecular mechanism has been proposed to explain the reduced enzyme's activity after MeHg exposure. Consistent with this hypothesis, cultured cells showed that MeHg induces a "selenium-deficient-like" condition, which affects GPx1 synthesis through a posttranscriptional effect (Usuki et al., 2010). Collectively, these effects point to the selenoenzyme GPx as an important molecular target in MeHg-induced neurotoxicity, which seems to be affected by MeHg earlier than other thiol-containing proteins. This effect, which is probably related to the higher nucleophilicity and softness of selenols when compared to thiols, reinforces the idea that the selenium-mercury interaction possesses an important role in the neurotoxicity induced by MeHg. In agreement with the foregoing data on MeHg and GPx, thioredoxin reductase (TrxR), another important antioxidant selenoprotein, has also been reported to be a molecular target involved in MeHg toxicity (Carvalho et al. 2008; 2011; Wagner et al., 2010). Based on both *in vivo* and *in vitro* experiments, the authors propose that MeHg can bind to selenocysteine residues present in the catalytic site of TrxR, thus causing enzyme inhibition that can compromise the redox state of cells.

Taking advantage of the Se-Hg interaction in biological systems, experimental studies have been directed at the potential protective effects of organic selenocompounds against MeHg–induced toxicity (Farina et al., 2003a, Farina et al., 2003b; Moretto et al., 2005a; Moretto et al., 2005b; de Freitas et al., 2009). These studies were performed based on the observation that organic selenocompounds, such as ebselen and diphenyl diselenide, are metabolized *in vivo* to selenol-containing intermediates, representing potential candidates to interact with MeHg in a high affinity way, thus minimizing its interaction with thiol-containing biomolecules. The development of studies on the search for potential therapeutic/antidotal strategies against MeHg toxicity is motivated by the fact that there are *no* effective treatments available which completely abolish the toxic effects of MeHg. Studies based on *in vitro* protocols showed that ebselen, an organic selenium compound, prevented MeHg-induced inhibition of glutamate uptake into rat cortico-cerebral slices (Moretto et al., 2005a). Ebselen also prevented MeHg-induced inhibition of glutamate uptake under *in vivo* conditions (Farina et al., 2003a), corroborating the *in vitro* data (Moretto et al., 2005a). More recently, an experimental *in vitro* approach showed that ebselen protected against MeHg-induced inhibition of glutamine uptake and mitochondrial collapse in primary cultures of rat astrocytes (Yin et al., 2011). It is noteworthy that ebselen has been reported to offer borderline but significant efficacy against excitotoxicity in Phase III studies, when the neurological outcomes of patients were determined in a short period after the neuropathological insult (Yamaguchi et al., 1998; Lee et al., 1999).The neuroprotective

activity of ebselen has been attributed to its GPx-like activity (Suzuki, 2009). However, clinical studies on ebselen are practically absent within the last decade. This most likely reflects the potential toxicity of this compound in relatively high levels, observed under in vitro and in vivo conditions (Yang et al., 2000; Farina et al., 2004). Alternatively, it is possible that the neuroprotective effects of ebselen observed close to the ischemic episodes could not be confirmed after a more prolonged longitudinal follow-up of the patients. Nevertheless, it is reasonable to suggest that when used in low dosages it may serve as an efficient antidotal therapy in MeHg poisoning due to the high affinity of MeHg for its selenol groups. In addition to ebselen, another interesting and promising organic selenocompound is diphenyl diselenide. This compound presents GPx-like activity and has been reported to present significant beneficial effects in several experimental pathological conditions (for a review, see Nogueira et al., 2004; Nogueira and Rocha, 2010). With particular emphasis on the toxicity elicited by MeHg, an *in vivo* experimental study showed that diphenyl diselenide administration was able to decrease the mercury levels in the liver, kidneys and brain of MeHg-treated mice (de Freitas et al., 2009), suggesting that it possesses direct chelating effects. Such effects are responsible, at least in part, for the increased excretion of MeHg from the body.

In summary, organic selenocompounds represent an interesting class of molecules for further investigation as potential therapeutic agents to treat MeHg poisoning. This is most likely a consequence of their ability to interact with endogenous and exogenous thiols to form “selenol intermediates”, which possess GPx-like activity (Nogueira et al., 2004) thus forming stable complexes with MeHg, rendering them more excretable. Additionally, ebselen and diphenyl diselenide can be reduced by TrxR, which generates the selenol of ebselen and selenophenol, respectively (Zhao et. 2002; Freitas et al. 2010), the nucleophylic intermediates that can bind to MeHg. Although these selenocompounds have been reported to display beneficial effects against MeHg-induced neurotoxicity in experimental studies, further research is necessary to evaluate the efficacy and safety of such compounds in humans.

Concluding remarks

Since the occurrence of the first outbreaks of MeHg poisoning in humans, the development of experimental studies has been instrumental in advancing the understanding of its metabolism and toxicokinetics, as well as the molecular mechanisms involved with its neurotoxic effects. Studies on the behavioral profile of MeHg-exposed animals have corroborated the clinical symptoms observed in humans poisoned by this toxicant. Moreover, the behavioral changes caused by MeHg exposure in animals (mainly represented by motor impairments) have been closely correlated to the biochemical alterations observed in these animals. *In vitro* and *in vivo* experimental approaches using cultured cells or living animals were also instrumental in demonstrating that MeHg entered the CNS complexed with the amino acid cysteine. Since this complex is structurally similar to methionine, MeHg is able to enter in neural cells mainly through the L-type neutral amino acid carrier transport (LAT) system by a mimicry mechanism. In addition, experimental studies were important in showing that MeHg is transported across the placenta from pregnant animals to their fetuses. Based initially on *in vitro* studies, the molecular mechanisms related to MeHg-induced neurotoxicity were partially elucidated. In this regard, glutamate and calcium dyshomeostasis, as well as ROS generation, are three important and interrelated phenomena that mediate a toxic cycle that culminates in neuronal death. These events were also proven to occur in *in vivo* conditions. Moreover, experimental studies were important in showing that MeHg reacted with selenol groups with high affinity, and that MeHg’s interaction with selenoproteins, such as the antioxidant enzymes glutathione peroxidase and thioredoxin reductase contribute to the increased oxidative stress. Finally, experimental studies have

shown that organic selenocompounds, such as ebselen and diphenyl diselenide, represent promising molecules that counteract the toxic effects elicited by MeHg, given their GPx-like activities and their potential chelating effects after biotransformation to selenol-containing molecules. Although experimental studies have been highly instrumental in shedding novel information on aspects involved in MeHg-induced neurotoxicity, further research on the role of microglial cells and neuroinflammation, as well as potential therapeutic/antidotal strategies is warranted.

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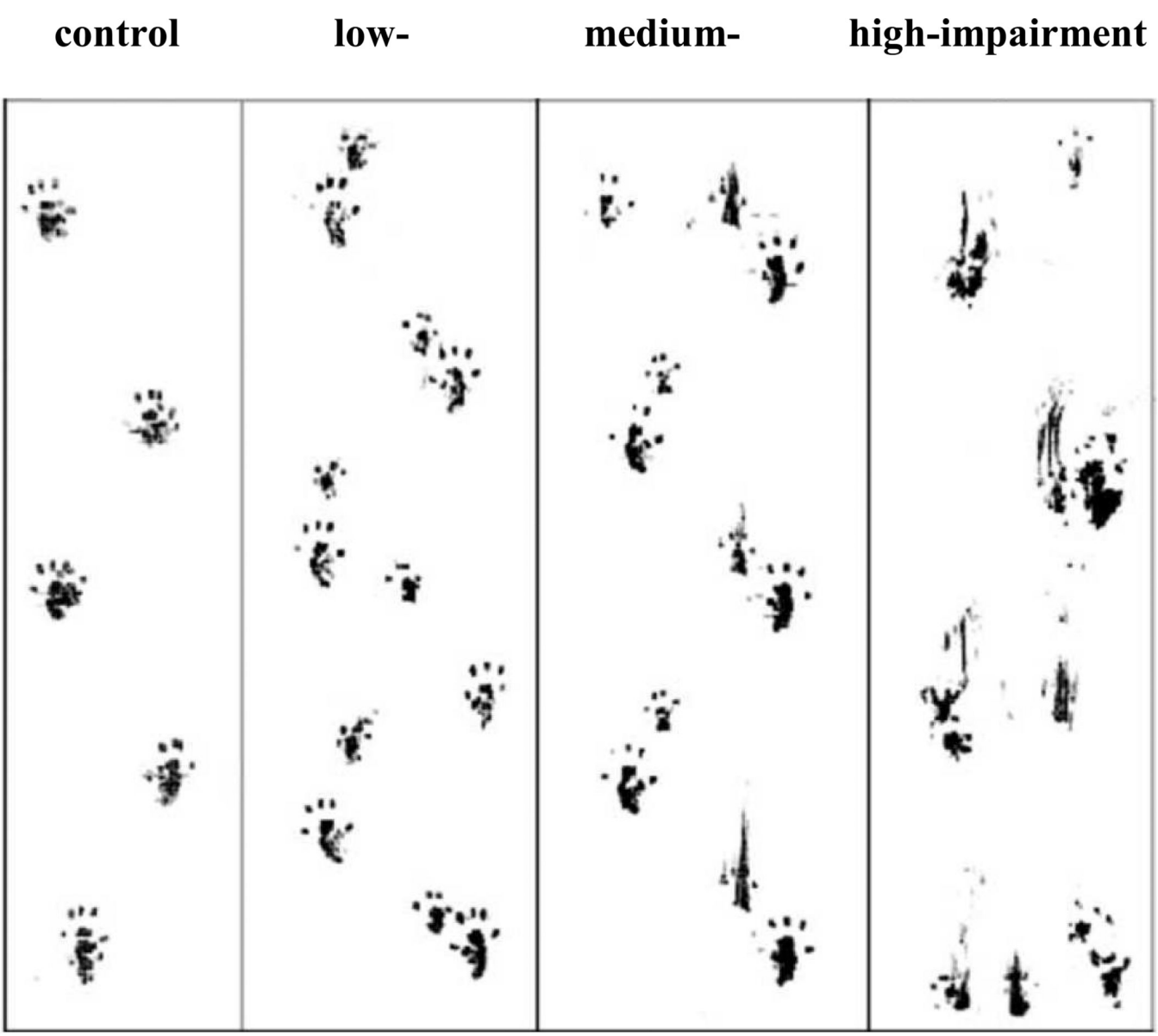

Figure 1. Progressive motor impairment in MeHg exposed mice

Animals were exposed to methylmercury (CH_3Hg^+; MeHg) (40 mg/L, diluted in drinking water) (Dietrich et al., 2004). The figure represents a footprint test evaluation in a randomly selected animal. Low-, medium- and high-impairment represent footprint tests performed at 7, 14 and 21 days after the beginning of the treatment, respectively.

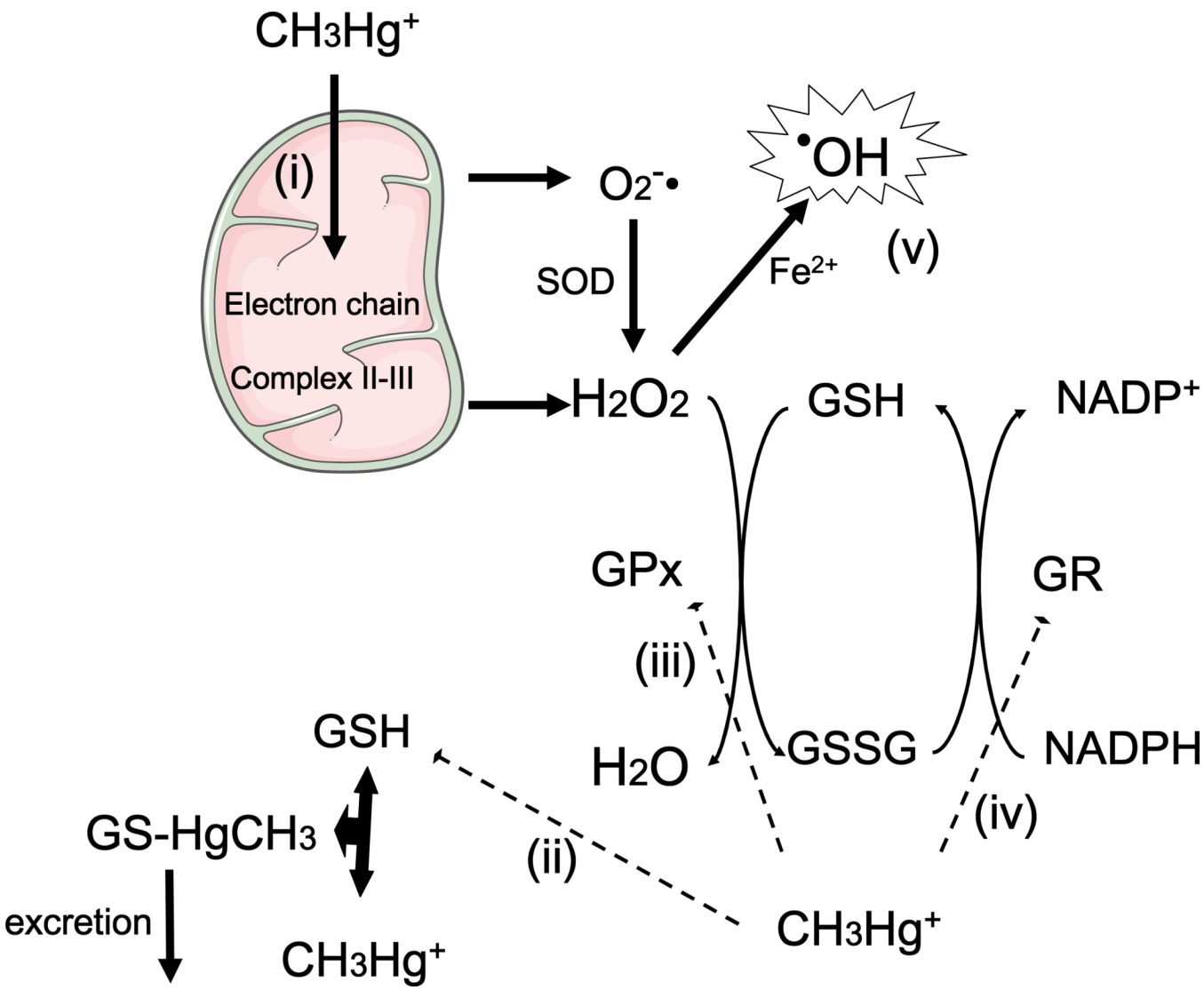

Figure 2. Effects of MeHg on the GSH antioxidant system

(i) Methylmercury (CH_3Hg^+; MeHg) disrupts mitochondrial electron transport chain, leading to increased formation of reactive oxygen species, such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2•−) (Franco et al., 2007; Mori et al., 2007). (ii) MeHg also reacts with reduced glutathione (GSH), leading to GSH depletion due to the formation of a MeHg–GSH ($GS–HgCH_3$) complex, which is excreted from the body. MeHg hampers the physiological increase in glutathione reductase (GR) and glutathione peroxidase (GPx) activities in the rodent CNS during the early postnatal period (iii and iv) (Stringari et al., 2008), but also decreases GPx activity in adult animals (Farina et al., 2003a, Franco et al., 2009). All these events (i–iv) culminate in increased ROS generation and oxidative stress (v).

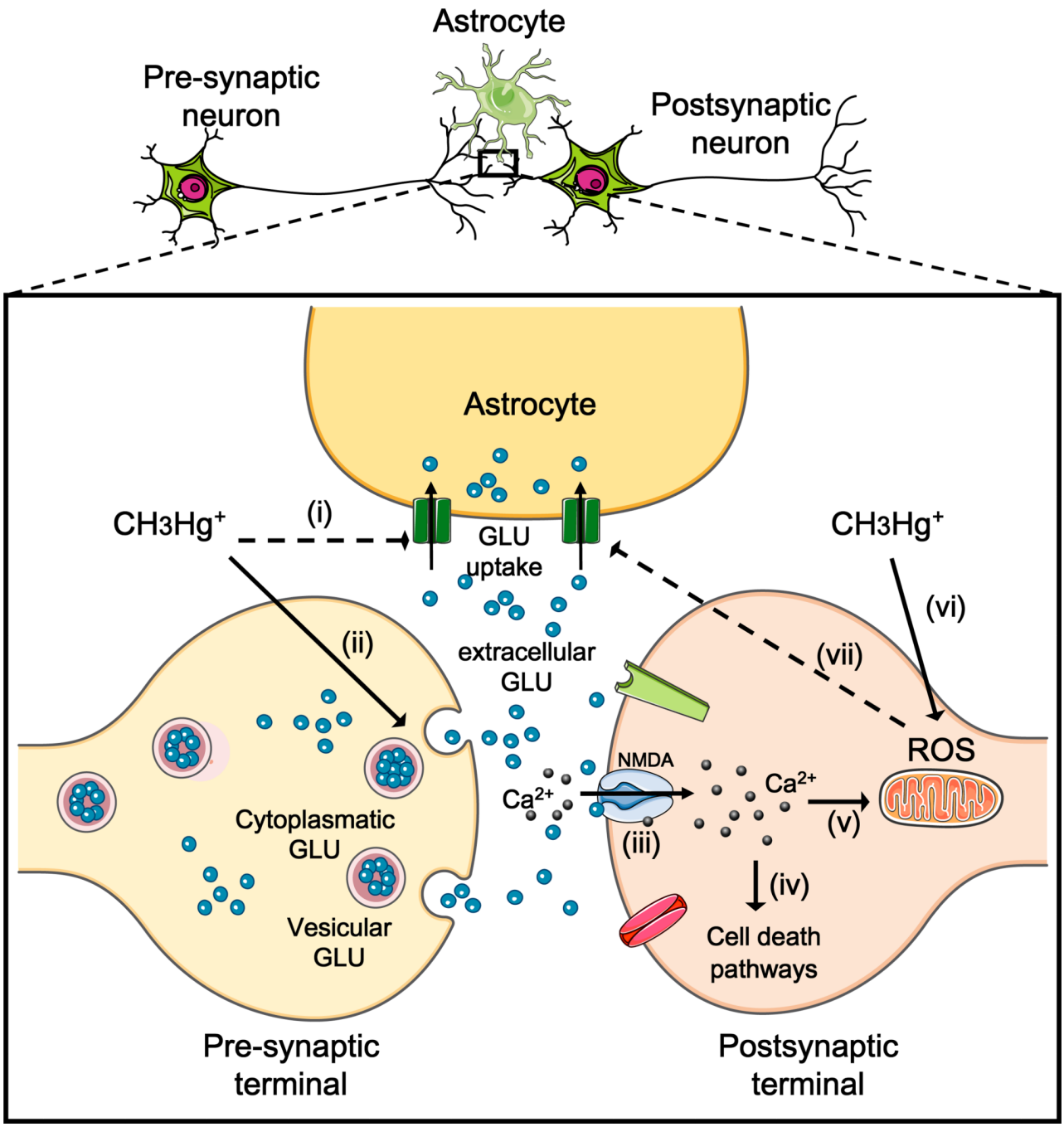

Figure 3. Interrelated association among MeHg-induced oxidative stress, Ca^{2+} and glutamate dyshomeostasis

The figure shows a tripartite synapses, where methylmercury (CH_3Hg^+; MeHg) inhibits astrocytic glutamate uptake (i) and increases glutamate release (ii), leading to elevated extracellular glutamate levels. High levels of extracellular glutamate overactivate *N*-methyl D-aspartate (NMDA)-type glutamate receptor (iii). Overactivation of NMDA glutamate receptors leads to increased influx of Ca^{2+} into postsynaptic neurons, causing activation of cell death pathways (Hidalgo and Donaso, 2008). Alternatively, Ca^{2+} taken up by mitochondria may cause mitochondrial dysfunction and increased reactive oxygen species (ROS) generation (v). This last event is also directly stimulated by MeHg (vi), which seems

to be related to misbalance in the electron transport chain (Mori et al., 2007). Increased levels of ROS (mainly H_2O_2) can directly decrease astrocytic glutamate uptake (vii) (Allen et al., 2001), contributing to excitotoxicity. GLU = glutamate.