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Methylmercury and brain development: A review of recent literature

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Abstract

Methylmercury (MeHg) is a potent environmental pollutant, which elicits significant toxicity in humans. The central nervous system CNS) is the primary target of toxicity, and is particularly vulnerable during development. Maternal exposure to MeHg via consumption of fish and seafood can have irreversible effects on the neurobehavioral development of children, even in the absence of symptoms in the mother. It is well documented that developmental MeHg exposure may lead to neurological alterations, including cognitive and motor dysfunction. The neurotoxic effects of MeHg on the developing brain have been extensively studied. The mechanism of toxicity, however, is not fully understood. No single process can explain the multitude of effects observed in MeHg-induced neurotoxicity. This review summarizes the most current knowledge on the effects of MeHg during nervous system development considering both, *in vitro* and *in vivo* experimental models. Considerable attention was directed towards the role of glutamate and calcium dyshomeostasis, mitochondrial dysfunction, as well as the effects of MeHg on cytoskeletal components/regulators.

Keywords

Methylmercury; developmental neurotoxicity; mechanisms

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1. Introduction

Mercury (Hg) is a heavy metal present in the environment that originates from from both natural and anthropogenic sources [1]. It occurs in nature as both inorganic (elemental or Hg^0, Hg^+ or Hg^{2+}) and organic compounds, such as ethylmercury (EtHg) and methylmercury (MeHg) [2]. MeHg is produced by biomethylation of inorganic mercury present in aquatic sediments, a reaction catalyzed primarily by aquatic microorganisms [3, 4]. It accumulates up the aquatic food chain, and reaches maximal concentrations in long-lived, predatory fish such as tuna, swordfish, shark and whale [1, 3]. As a consequence, populations whose diet consists largely of fish and seafood may be potentially exposed to high levels of MeHg, thus rendering these communities vulnerable to toxicity [2, 5].

MeHg has an extensive toxicological history, with adverse effects in several organ systems observed throughout human life span [1, 6]. As mentioned above, seafood represents a major source of MeHg, and about 95% of that ingested is absorbed in the gastrointestinal tract [7]. After absorption, MeHg is ubiquitously distributed, and readily penetrates the CNS [8]. The brain has high affinity for MeHg, and concentrations can be 3–6 times greater than that in blood [6]. MeHg can distribute to all brain regions by crossing the blood-brain barrier via the neutral amino acid transport system L as a complex with L-cysteine [9].

It is well documented that the developing CNS is particularly vulnerable to MeHg [10–12]. Taking that into account, this review summarizes the current knowledge regarding the molecular mechanisms involved in MeHg-induced developmental neurotoxicity. Considerable attention has been directed towards the role of glutamate and calcium dyshomeostasis, mitochondrial dysfunction as well as the effects of MeHg on cytoskeletal components/regulators.

1.1. Neurotoxic Effects of Methylmercury – General Aspects

The CNS is a major target of MeHg [13]. In adults (i.e. the mature CNS) toxic effects of MeHg are characterized by a long latency period before the appearance of symptoms [3]. The initial symptoms include blurred vision, weight loss, paresthesias of the circumoral area and hands and feet, followed by visual-field constriction, ataxia and psychiatric sympotmatology [3, 14]. Furthermore, in adults the signs and symptoms of MeHg poisoning are associated with loss of neuronal cells in specific brain regions, such as the visual cortex and the cerebellum [15]. Post-mortem histopathological analysis has shown a significant loss of cerebellar granule cells (CGC) with relative preservation of the adjacent Purkinje cell layer [16].

The relationship between MeHg-induced motor deficit and cerebellar damage is, in fact, a well-described phenomenon [17]. Several studies have shown that exposure to MeHg can produce behavioral deficits related to locomotor activity and motor performance in adult mice [18–21]. Experimental studies conducted in the last four decades have contributed to the current understanding of pivotal events that mediate MeHg-induced neurotoxicity. Disruption of calcium (Ca^{2+}) homeostasis or induction of oxidative stress via overproduction of reactive oxygen species (ROS) or reduction of antioxidant defenses are likely to be critical factors in MeHg-induced cell damage, as are interactions with sulfhydryl (-SH)

groups [5, 22–25]. The high affinity of MeHg for -SH groups on the amino acids cysteine and methionine may alter the structure of a large number of proteins and lead to disruption of various intracellular functions. A number of molecular targets and mechanisms have been implicated in both *in vitro* and *in vivo* studies and no single mechanism is likely to be explanatory [22, 26, 27].

In the CNS, MeHg can adversely affect many cell types, and a large body of evidence suggests that astrocytes represent a preferential site for its accumulation, playing a crucial role in MeHg neurotoxicity [28–30]. Astrocytes have an essential role in brain development, function, and plasticity. These cells coordinate neuronal development and survival, control synapse formation and function, and play a major role in the formation of neuronal circuits [31–34]. Moreover, astrocytes carry out other critical functions in the brain, including formation of the blood brain barrier and expression of neurotransmitter transporters [35]. MeHg interferes with glutamate and aspartate uptake in astrocytes, increasing glutamate concentration in the synaptic cleft, resulting in high levels of extracellular glutamate, which is toxic to neurons [25, 36–38]. Furthermore, MeHg inhibits the astrocytic uptake of cystine and cysteine, compromising glutathione (GSH) synthesis and cellular redox status [39–41]. In addition to its effects in astrocytes, MeHg can also stimulate the production and secretion of lysosomal proteases in microglial cells, also leading to neuronal toxicity [42].

1.2. Effects of MeHg on the Developing Brain

Prolonged pre and/or perinatal exposure to MeHg, even at moderate doses, is related to multiple deficits in neurons and glia including abnormal migration, differentiation and growth [43, 44]. The developing brain is extremely sensitive to MeHg poisoning. In fact, children exposed to MeHg may exhibit decreased IQ, impaired movements, visuospatial perception, and speech, a medical phenomenon known as fetal Minamata disease (FMD) [45–47]. The effects of developmental exposure to MeHg on animal behavior include, among others, reduced motor activity [52] and a decrease in memory [53–55] and learning [56]. Studies modeling *in utero* human exposures in postnatal rat pups, show that MeHg can alter the expression of many developmental regulators and genes involved in small GTPase signaling pathways regulating cell growth and proliferation [57]. Furthermore, several reports have shown that MeHg can induce mitotic arrest and caspase-dependent apoptosis in the developing CNS [27, 58–60], suggesting that proliferative cell populations may be especially vulnerable to MeHg-induced cell death.

In 1952, the first case of congenital MeHg poisoning was described in Sweden. Children from a family that used flour made from a MeHg-contaminated seed grain, were found to have impaired intellectual function and motor development [61]. Some years later, a catastrophic epidemic from environmental MeHg contamination was reported in Minamata, Japan. The ingestion of contaminated fish by pregnant women evidently poisoned the fetuses, and consequently many children born from 1955 onward had neurodevelopmental disturbances [62]. More recently, studies conducted in human populations found an association between maternal MeHg exposure during pregnancy, and neurological and neuropsychological deficits in children [63–65].

Characteristics and mechanisms of MeHg poisoning cases in adults are distinct from that in children [15]. In the neonatal period or at early ages, the damage caused by MeHg is more widely distributed throughout the CNS [3, 12, 66]. The injury is pervasive, and often, the earlier the exposure, the more generalized the damage observed [66]. Experimental data have provided evidence that MeHg can affect processes that are extremely important to brain development, such as: (i) interaction with elements of the cytoskeleton that consequently affect the movement of neuronal cells from the site of germination to the final site of function [67, 68] (ii) interference with the formation of dendrites and axons (i.e. interneuronal contacts) [68] and (iii) induction of apoptosis, which occurs normally during the development of the brain [69, 70].

The next section provides an overview of recent findings regarding MeHg-induced developmental neurotoxicity, as well as a discussion about the potential molecular mechanisms of MeHg-induced cell death that have recently been described in the literature using animal and cell culture models.

2. Mechanisms of MeHg-induced Developmental Neurotoxicity

2.1. Glutamate and Ca^{2+}

It has been demonstated that MeHg-induced neuronal death can occur either by necrosis [71–73] or apoptosis [60, 70, 71, 73–76], depending on the level of exposure, the cell type, and the cellular defense mechanisms. In many instances, such effects are mediated by disruption/modulation of cellular Ca^{2+} homeostasis or increased generation of ROS in the mitochondria or other intracellular compartments [77]. In fact, Ca^{2+} is known to play a critical role in cell loss in the CNS and Ca^{2+} overload has been shown to trigger either necrotic or apoptotic cell death [for review, see Orrenius et al., 2003 - [78]. Sustained elevated Ca^{2+} levels have been found in various cell types after MeHg exposure, and protective effects of Ca^{2+}-chelators or Ca^{2+}-channel blockers have been reported *in vitro* and *in vivo* [79–84]. Importantly, MeHg at low micromolar concentrations causes prolonged increases in intracellular, cytosolic Ca^{2+} concentrations [79, 80, 85, 86], which may be secondary to increases in extracellular glutamate [25]. A number of experimental findings point to inhibition of glutamate uptake by astrocytes, increase in spontaneous release of glutamate from presynaptic terminals, and inhibition of vesicular glutamate uptake as critical phenomena linked to MeHg-mediated excitotoxicity [25, 41, 87]. During critical CNS developmental windows, MeHg-induced disruption of the glutamate pathway may interfere with cell proliferation and cell fate decisions. Using a MeHg drinking water exposure paradigm in mouse dams, Manfroi et al. [88] investigated the exclusive contribution of MeHg exposure through maternal milk. This report demonstrated that the offspring of the exposed dams exhibitied glutamate uptake inhibition in the cerebellum; this was not observed in the dams, however. Experimental data obtained in cortical cell cultures from pups exposed to MeHg during gestation (8 mg/kg, gestational-days 8 or 15), showed effects on glutamatergic neurotransmission and increased responsiveness of the N-methyl-D-aspartate (NMDA)-type glutamate receptors. The basal extracellular glutamate levels measured in these cultures was higher than those measured in cultures obtained from control pups [53]. This suggests an enhanced sensitivity of NMDA receptors, which could make

premature cortical neurons more susceptible to MeHg neurotoxicity. Interestingly, MK-801, a non-competitive antagonist of NMDA receptors, administered intraperitoneally with MeHg during brain development, markedly ameliorated MeHg-induced neurodegeneration [89]. All the above mentioned observations are consistent with MeHg increasing extracellular glutamate levels. Overactivation of NMDA receptors increases Ca^{2+} influx into neurons, therefore leading to activation of important pathways involved with cell death. Alternatively, Ca^{2+} can be taken up by mitochondria, where it may stimulate ROS production [90]. In this regard, MeHg induces pro-oxidative damage in the developing CNS, by disrupting postnatal development of the glutathione-antioxidant system [91]. Furthermore, it has been shown that exclusive lactational exposure to MeHg causes neurotoxicity in pups by decreasing glutamate uptake into cerebellar slices and increasing oxidative stress [88, 92].

Neurons in patients with FMD are hypoplastic, ectopic, and disoriented, indicating disrupted migration, maturation, and growth. As previously mentioned, the cerebellum is one of the most susceptible brain regions to MeHg exposure, and profound loss of cerebellar granule cells (CGCs) is detected in the brains of patients with FMD [93]. MeHg causes a characteristic lack of CGC migration, and subsequently laminar cortical organization in the developing cerebellum *in vivo* [66, 93]. Significant impairment of CGC migration by MeHg has been shown *in vitro* [94] and in organotypic slice culture under acute and/or high level [67] or repeated exposure to low micromolar concentrations, as well [95]. The question of which signaling molecules are involved in these effects on neuronal cell migration, however, remains to be determined. Among the early effects of MeHg in CGCs is a pronounced increase of Ca^{2+}, consisting of an initial release of stored intracellular Ca^{2+} followed by influx of extracelular Ca^{2+} [79, 96]. This effect occurs at much lower MeHg concentrations in CGCs than in other, less sensitive cells [97, 98]. It has been shown that MeHg-induced neuronal death is attenuated by chelating Ca^{2+} *in vitro* [80], while *in vivo*, the neurological signs of MeHg toxicity in rats are attenuated by Ca^{2+} channel blockers [82], suggesting that loss of Ca^{2+} homeostasis is critical to MeHg-induced death of CGCs. Nevertheless, a study conducted in CGCs exposed to nanomolar concentrations of MeHg (300 nM) for extended periods of time (4 days *in vitro*), revealed that the Ca^{2+} concentration remained unchanged, while a significant reduction of glutathione peroxidase-1 (GPx-1) activity was observed [99]. GPx-1 is a crucial antioxidant enzyme involved in neuronal detoxification of hydrogen peroxide [100]. Interestingly, the authors showed that GPx-1 inhibition occurred before any changes on potential targets of MeHg toxicity, and GPx1 overexpression was able to prevent MeHg-induced neuronal death in CGCs [99]. Recently, it has been proposed that alterations of Ca^{2+} spike frequency could be one of the primary targets of MeHg-induced inhibition of granule cell migration, in a mouse model of FMD. The authors showed that MeHg slows the speed of granule cell migration and reduces the frequency of spontaneous Ca^{2+} spikes in granule cell somata in a dose-dependent manner [93]. Ca^{2+} spikes (or transient Ca^{2+} elevations) are known to play an essential role in maintaining the movement of cells ranging from fibroblasts to immature neurons [101–103]. Thus, the inhibition of Ca^{2+} spikes by buffering of intracellular Ca^{2+} levels reduces or inhibits cell movement [104].

Alterations in voltage-sensitive Ca^{2+} channels (VSCCs) have also been reported in MeHg-induced neurotoxicity. It is known that Ca^{2+} channels are expressed early during development in many parts of the CNS. These channels participate in many intracellular

events such as neurotransmitter release, axonal growth, and gene expression by allowing influx of extracellular Ca^{2+}, resulting in the subsequent activation of proteins [105, 106]. Neonatal CGCs in culture possess a rich diversity of Ca^{2+} channels, with five postulated types (L, N, P, Q, and R) being described to date [107]. Migration of CGCs, which is inhibited by MeHg exposure, is dependent on N-type VSCCs [108, 109]. It has been reported that Ca^{2+} channels are a likely MeHg target [81, 110, 111] because of their location on the plasma membrane and strict regulation by various intracellular mediators [81]. Acute and chronic effects of MeHg on VSCCs have been characterized in PC12 cells [110–112], a well characterized model for differentiated neurons. In neonatal CGCs, a more sensitive cell type, this toxicant can inhibit VSCC function even at submicromolar concentrations [81].

A recent study reported that acute exposure to MeHg causes developmental stage-dependent increases in Ca^{2+} concentration in slices of neonatal rat cerebellum. The effects of MeHg were most prominent in CGCs during development or early stages of migration. In addition, the authors showed that the effects of MeHg on CGCs during migration is attenuated by gamma-aminobutyric acid ($GABA_A$) receptor modulation, indicating that this receptor can be affected in any stage of CGC development [113]. In fact, studies conducted in hippocampal and cerebellar slice preparations revealed that MeHg can impair inhibitory GABAergic neurons [114–116]. Moreover, when compared with glutamatergic synaptic transmission, hippocampal GABAergic transmission seems to be more sensitive to the effects of MeHg [116]. The effect of several developmental neurotoxicants on mRNA levels of various neural markers was analyzed in CGCs, and results revealed that prolonged exposure to 50 nM MeHg resulted in a significant downregulation of $GABA_A$ receptor mRNA, while other neural markers were unchanged [117].

2.2. Mitochondria

Mitochondria represent the major sites for oxidative stress, and are critical for regulation of neuronal cell death induced by MeHg via Ca^{2+} dyshomeostasis and/or ROS generation [78–80]. Indeed, this organelle has been considered the main source of intracellular Ca^{2+} release in CGC cultures upon MeHg exposure [96]. Previous studies suggest that MeHg acts via a mitochondrial-dependent cascade increasing Bax, cytosolic cytochrome c, and caspase-9, to elicit cell death in primary neuronal precursors and cell lines [76]. Acute MeHg exposure was able to increase the levels of activated caspase-3 in the postnatal rat hippocampus, and cause subsequent juvenile learning deficits [59, 27]. Furthermore, exposure of the developing hippocampus *in vivo* induced mitochondrial-dependent cell death via sequential activation of caspase-9 and caspase-3 [60]. In fact, it is well known that apoptotic cell death occurs via activation of well characterized biochemical pathways, with caspase-8 and caspase-9 acting as initiator caspases that cleave and activate caspase-3, which is normally considered one of the last steps in cell death [118]. In differentiating PC12 cells, immunocytochemical analysis showed that activated caspase-3-positive neurons were significantly increased 1 day after exposure to 100 nM MeHg. Importantly, it was also observed apoptosis preceded inhibition of neurite extension [119]. In the developing brain, caspase-3 has been found to play a crucial role not only in apoptosis [120], but also in nonapoptotic neuronal functions, including neural stem cell differentiation [121].

In rats, peak of postnatal neurogenesis (postnatal day 7– P7) corresponds to human hippocampal development occurring during the third trimester of gestation. Deficits in hippocampal-dependent behavioral tasks might be a sign of impaired neurogenesis [122]. MeHg exposure in P7 rats led to reductions in cell cycle regulator cyclin E, as well as induction of mitochondrial-dependent apoptosis by activation of caspase-3 [59, 60]. Rats exposed to a single injection of 5μg/g MeHg at P7 showed profound juvenile spatial learning impairment and decreases in cell number in P21 hippocampus, that correlated with acute caspase-dependent apoptosis [27]. Furthermore, acute exposure to an environmentally relevant dose of MeHg (0.6μg/g, 24h) in P7 rats, induced caspase-3 activation in neural stem cells (NSCs) of the dentate gyrus (DG), and caused hippocampal-dependent memory deficits during adolescence, assessed by Morris water maze [123]. In addition, a significant degeneration of neuritic processes, and either apoptotic or necrotic death was observed in cortical cultures obtained from pups born to 8 mg/kg MeHg-exposed dams, which was correlated with long term memory impairment of adult offspring subjected to a memory task [73].

While pro-apoptotic signals stimulated by MeHg may involve caspase-3 activation, the overall process is likely to be more complex. For example, ROS-induced mitochondrial dysfunction causes the release of Ca^{2+} into the cytosol, which can trigger the activation of calpains, a calcium-dependent protease. A number of *in vitro* studies performed in CGCs and hippocampal cells after MeHg treatment reported mitochondrial dysfunction (determined by cytochrome C release) and Ca^{2+} influx in association with increased calpain protein level [124–126]. Calpains along with caspases belong to the family of cysteine proteases, which play important roles in various necrotic and apoptotic conditions [127]. This pathway is activated by the perturbation of intracelular Ca^{2+} homeostasis[128], which can occur during exposure to various toxic stimuli, such as MeHg. Calpains are known to regulate the activity of several protein kinases and phosphatases that modify the cytoskeleton, in addition to cleaving diverse cellular substrates including cytoskeletal proteins directly [129, 130], as well as tubulins. Very low levels of MeHg (30nM) can induce apoptotic cell death of cultured rat cerebellar neurons via increased intracellular Ca^{2+} concentration and calpain activation [125]. It has been shown that calpains are activated in MeHg-exposed NSCs [76], with an associated increase in intracelular Ca^{2+} as observed in other apoptotic models [131, 132]. Interestingly, MeHg-exposed NSCs undergoing apoptosis showed activation of two parallel pathways involving caspases and calpains, as proven by either the partial protection exerted by the caspase (zVAD-fmk) or calpain (E64d) inhibitor alone, or the full protective effect of the two inhibitors together [76].

2.3. Cytoskeleton

The architecture and survival of neurons depends significantly upon their cytoskeletal components, specifically actin filaments and microtubules, such as β-tubulin. Microtubules are involved in a variety of cell functions important for the development and maintenance of cell shape, reproduction and division, signaling, intracellular transport, and motiliy. In this way, any disturbance of these dynamic structures or their polymerization/depolymerization status could compromise cell survival [133, 134]. MeHg has long been known to be a potent inhibitor of microtubule polymerization [135–137]. In vitro studies demonstrated that MeHg

has high affinity for tubulin sulphydryl groups (-SH), depolymerizing cerebral microtubules, and directly inhibiting their assembly [135, 137]. Microtubule fragmentation and neuronal network dissolution have been observed in cultured primary cerebellar granule neurons exposed to concentrations of MeHg as low as 0.5–1 μM. Such effects occurred early, and preceded the onset of apoptotic signs in this *in vitro* model [71]. More recently, a proteomic study of CGCs exposed to MeHg (≤ 1μM) for several days revealed that MeHg can disrupt the balance between phosphorylated and non-phosphorylated cofilin forms [138]. The balance of cofilin phosphorylation/dephosphorylation is a central component of actin dynamics in migrating cells and facilitates actin filament turnover, which is responsible for neuron shape, migration, polarity formation, and regulation of synaptic structure and function [139].

The Rho/ROCK signaling pathway, one of the most well-studied Rho family GTPase-effector pathways, is the major regulator of stress fiber formation under most physiological conditions. This pathway is closely related to the pathogenesis of several CNS disorders, and involved in many aspects of neuronal function, including neurite outgrowth and retraction [140]. Among Rho family proteins Ras-related C3 botulinum toxin substrate 1 (Rac1) and cell division cycle 42 (Cdc42) are known to promote neurite extension and inhibit apoptosis [141]. Moreover, the Rho-associated protein kinases (ROCKs) are central regulators of the actin cytoskeleton downstream of the small GTPase Rho [142]. Along this line, a study reported downregulation of Rho-family proteins Rac1 and Cdc42 in early stages of MeHg-induced cytotoxicity. The authors demonstrated that neuritic degeneration precedes MeHg-induced apoptotic cell death in cortical neurons [143]. The therapeutic effects of Rho/ROCK inhibitors have been demonstrated in some *in vitro* and *in vivo* experimental models, and this pathway has become an attractive target for the development of drugs for treating CNS disorders [144]. In this regard, treatment of cultured cortical neuronal cells with Rho/ROCK inhibitors suppressed MeHg-induced neuronal degeneration and apoptotic cell death at 100 nM MeHg for 3 days. Furthermore, the Rho/ROCK inhibitors partially prevented MeHg-induced toxicity in a rat model (20 ppm MeHg in drinking water for 28 days) [145].

2.4. Cell Differentiation

Normal differentiation of neurons and glial cells during development is crucial in neurogenesis, which is particularly sensitive to environmental toxicants like MeHg. In this regard, *in vitro* cultures of NSCs provide an appropriate model for studying the adverse effects of MeHg and other developmental neurotoxicants on survival, proliferation, differentiation, and migration. NSCs are self-renewing cells, that play an essential role in the development and maturation of the CNS, with potential to differentiate into neurons, astrocytes, and oligodendrocytes [146]. Nanomolar (2.5–10 nM) concentrations of MeHg can affect proliferation and differentiation of rodent embryonic NSCs, and the activation of Notch signaling seems to be involved in this process [147]. In fact, constitutive expression of Notch protein is reported to inhibit neurite outgrowth in PC12 cells [148]. Moreover, it has been shown that low concentrations of MeHg (2.5–25 nM) reduce neural progenitor cell proliferation [76, 149–151], expression of genes related to cell cycle regulation (cyclin E, p16 and p21) [149, 151, 140], cellular senescence, and mitochondrial function (repression of the mitochondrial respiratory chain enzymes complexes I and III) [149]. Interestingly, the

alterations detected in cell proliferation, cell cycle regulation (p16 and p21 regulators), and mitochondrial function, were observed in cells directly exposed to MeHg and in their daughter cells (from passages 2 and 3) cultured under MeHg-free conditions, suggesting that the effects of MeHg on NSCs are heritable [149].

3. Conclusion

MeHg is a hazardous environmental pollutant, of great concern to public health because of its toxicity to the CNS. Exposure events have demonstrated that fetuses are much more sensitive to MeHg than adults. It has been reported that acute or chronic MeHg exposure can cause adverse effects during all developmental periods. Indeed, MeHg is now recognized as an important developmental neurotoxicant, and over the last decade, notable progress has been made in understanding its mechanism of neurotoxicity. One of the most widely documented effects caused by MeHg on the CNS is associated with glutamate-mediated excitotoxicity, which can be linked to or followed by intracellular Ca^{2+} overload. In fact, impairment of intracellular calcium and glutamate homeostasis, oxidative stress generation, as well as mitochondrial dysfunction are critical factors in MeHg-induced cell death. These connected phenomena create complexity in understanding the initial events of neurotoxicity. Current knowledge of the precise targets of MeHg is incomplete, and detailed experimental data regarding the mechanism of action, mainly during the neurodevelopmental period, are needed. In this review, the mechanisms implicated in the published literature are particularly pertinent to MeHg-induced developmental neurotoxicity. For instance, MeHg-induced negative effects on cytoskeletal proteins (microtubules) and cytoskeleton-regulating proteins (Rho-family proteins), thereby disturbing neuronal migration and differentiation. Relatively little new information, however, has been found about the potentially neurotoxic effects induced by interaction between MeHg and cytoskeletal proteins. In addition, the relationship between the Rho/ROCK pathway and MeHg neurotoxicity is still very limited, and consideration should be given to future research. This review has provided an overview of the main molecular mechanims involved in neurotoxicity induced by MeHg-exposure during the developmental period. Taken together, the data discussed in this paper collaborate for a better understanding of the multifactorial mechanisms involved in MeHg-induced cell damage, suggesting, a number of directions for future research.

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Table 1

In vivo studies on molecular mechanisms of MeHg-induced developmental neurotoxicity

Experimental model	Treatment	Tissue	Results	Reference
Swiss albino mice	Drinking water - 15 mg/l for 21 days	Cerebellum of suckling mice indirectly exposed to MeHg for 21 days	• Inhibition of glutamate uptake • Increased levels of hydroperoxide	[88]
Sprague–Dawley rats	Acute - intragastric intubation (8 mg/kg on GD 8 and 15)	Cortical cell cultures from pups exposed to MeHg during gestation	• Increased extracellular glutamate levels • Increased responsiveness of the NMDA receptors	[53]
Swiss albino mice	Drinking water - (1, 3 and 10 mg/l) during the gestational period	Whole brain of P1, P11 and P21 pups	• Dose-dependent inhibition of GSH levels • Dose-dependent inhibition of Gpx and GR activities • Increased F2-isoprostanes levels	[91]
Sprague–Dawley rat pups (P7)	Acute - subcutaneous injection (5 μg/g)	Hippocampus	• Increased levels of activated caspase-3 (24 h after injection) • Decreased cell number in P21 hippocampus	[27]
Sprague–Dawley rat pups (P7)	Acute - subcutaneous injection (5 μg/g)	Hippocampus	• Increased cytochrome c release • Increased Bax protein • Caspase-9 and caspase-3 activation	[60]
Sprague–Dawley rat pups (P7)	Acute - subcutaneous injection (0.6 μg/g)	NSCs from dentate gyrus hilus and granule cell layer	• Acute caspase activation • Decreased cell number (P21) • Reduced neural precursor proliferation (P21)	[123]

Abbreviations: GD (gestational days), P (postnatal days), NSCs (neural stem cells), Gpx (glutathione peroxidase), GR (glutathione reductase)

Table 2

In vitro studies on molecular mechanisms of MeHg-induced neurotoxicity

Experimental model	Exposure dose	Results	Reference
Primary cerebellar granule cells (Sprague–Dawley rats)	0.5 μM	• Increased $[Ca^{+2}]_i$ concentration • Mitochondrial membrane depolarization	[96]
Cerebellar slices from Sprague-Dawley rats (P8–P12)	10 – 20 μM	• Increased $[Ca^{+2}]_i$ concentration in CGCs	[113]
Mouse neuronal progenitor cell line (C17.2) Primary embryonic cortical NSCs (Sprague–Dawley rats)	0.25 – 0.5 μM 0.025 – 0.05 μM	• Bax activation • Cytochrome c release • Caspase activation • Calpain activation	[76]
PC12 (rat pheochromocytoma cell line)	0.1 μM	• Increased TUNEL-positive apoptotic nuclei • Caspase 3 activation • Inhibition of neurite extension	[119]
HT22 cells (mouse hippocampal cell line)	4.0 μM	• Decreased mitochondrial membrane potential and function • Reduced ATP levels • Cytochrome c release • Calpain activation • Lysosomal disruption	[126]
Primary embryonic cortical NPCs (Sprague–Dawley rat)	10 nM	• Cyclin E downregulation • GSK-3β upregulation • Reduced NPCs proliferation	[151]
Primary embryonic cortical NSCs (Sprague–Dawley rats)	2.5 – 5.0 nM	• Reduced NSC proliferation • Increased expression of p16 and p21 (cell cycle arrest) • Altered expression of mitochondrial genes	[149]
Primary embryonic cortical culture (Sprague–Dawley rats)	0.1 – 1 μM	• Neuritic degeneration • Caspase-dependent apoptosis • Rac1 and Cdc42 downregulation	[143]

Abbreviations: P (postnatal days), NSCs (neural stem cells), CGCs (cerebellar granule cells), NPCs (neural progenitor cells).