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# Effects of methylmercury on spinal cord afferents and efferents—A review

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ABSTRACT

Methylmercury (MeHg) is an environmental neurotoxicant of public health concern. It readily accumulates in exposed humans, primarily in neuronal tissue. Exposure to MeHg, either acutely or chronically, causes severe neuronal dysfunction in the central nervous system and spinal neurons; dysfunction of susceptible neuronal populations results in neurodegeneration, at least in part through $Ca^{2+}$-mediated pathways. Biochemical and morphologic changes in peripheral neurons precede those in central brain regions, despite the fact that MeHg readily crosses the blood-brain barrier. Consequently, it is suggested that unique characteristics of spinal cord afferents and efferents could heighten their susceptibility to MeHg toxicity. Transient receptor potential (TRP) ion channels are a class of $Ca^{2+}$-permeable cation channels that are highly expressed in spinal afferents, among other sensory and visceral organs. These channels can be activated in numerous ways, including directly via chemical irritants or indirectly via $Ca^{2+}$ release from intracellular storage organelles. Early studies demonstrated that MeHg interacts with heterologous TRP channels, though definitive mechanisms of MeHg toxicity on sensory neurons may involve more complex interaction with, and among, differentially-expressed TRP populations. In spinal efferents, glutamate receptors of the N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and possibly kainic acid (KA) classes are thought to play a major role in MeHg-induced neurotoxicity. Specifically, the $Ca^{2+}$-permeable AMPA receptors, which are abundant in motor neurons, have been identified as being involved in MeHg-induced neurotoxicity. In this review, we will describe the mechanisms that could contribute to MeHg-induced spinal cord afferent and efferent neuronal degeneration, including the possible mediators, such as uniquely expressed $Ca^{2+}$-permeable ion channels.



## 1. Introduction

### 1.1. Methylmercury (MeHg) neurotoxicity

Mercury is an environmental toxicant derived from both natural and anthropogenic sources. In the environment, elemental mercury ($Hg^0$) originates from the Earth's crust and volcanic emissions. Anthropogenic sources include burning of coal, waste incineration and small -scale gold mining. $Hg^0$ enters the atmosphere as a vapor, where it then becomes part of the water cycle. Once in the atmosphere, $Hg^0$ can remain in its vaporous state and move throughout the hemisphere, or become oxidized to $Hg^{2+}$ and recycled to the Earth in rain (Clarkson and Magos, 2006; Horowitz et al., 2014). Accumulated $Hg^{2+}$ is a substrate for methylation by sulfate-reducing bacteria in bodies of water,

*Abbreviations:* AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; DRG, dorsal root ganglia; ($[Ca^{2+}]_i$), intracellular $Ca^{2+}$ concentration; KA, kainic acid; MD, Minamata Disease; MeHg, methylmercury; MN, motor neuron; NMDA, N-methyl-d-aspartate; SOD1, superoxide dismutase-1; TRP, transient receptor potential.

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generating MeHg. Given its relative lipophilic properties, MeHg then enters the aquatic food chain through the process of bioaccumulation. Humans are exposed to MeHg primarily through the consumption of contaminated seafood or marine mammals (Clarkson, 1995). A relatively recent contributor to MeHg exposure and subsequent toxicity, is the use of mercury amalgam for extracting gold in artisanal gold mining (Schmidt, 2012). This may represent the most frequent contemporary route of exposure to MeHg, especially in South America (Ashe, 2012; Fraser, 2016) and Africa (Odumo et al., 2014; Rajaee et al., 2015).

MeHg is the most prevalent form of organic Hg encountered, and it is of environmental and public health concern due to its prevalence and persistence in the environment. Two major MeHg poisoning episodes triggered the many studies and subsequent discoveries regarding MeHg toxicity: Minamata, Japan (1953–1956) and Iraq (1970s) (Bakir et al., 1973; Clarkson and Magos, 2006; Eto, 1997). Studies from these two poisoning events led to the following findings relevant to this review: 1) there is a latent period between the exposure to MeHg and the onset of symptoms; 2) severity of symptoms is MeHg dose-dependent; and 3) the first and most prevalent symptom reported is paresthesia, followed, often in succession, by ataxia, muscle weakness, tremor, dysarthria, and hearing and visual impairment (Bakir et al., 1973; Eto, 1997). Patients presenting with these clinical signs and symptoms as a result of environmental exposure to MeHg were diagnosed with the neurologic syndrome referred to as Minamata Disease (MD) (McAlpine and Araki, 1958).

Developmental neurotoxicity to MeHg was especially apparent in these two major poisoning episodes (Bakir et al., 1973; Eto, 1997). However, MeHg exposure occurs over the life span for anyone consuming certain species of fish or marine mammals, and the consequences of chronic, low-level adult onset exposures are an underexplored area. Ongoing studies of chronic dietary exposure to MeHg in the Seychelle (Davidson et al., 2000) and Faroe Islands (Grandjean et al., 1999), in which the primary food source is fish and/or marine mammals have been following the development of neurological sequellae.

Another more recently discovered region under critical examination is the Peruvian Amazon (Ashe, 2012; Fraser, 2016; Gardner, 2012), where Hg is used extensively for artisanal gold mining. MeHg toxicity under these conditions is somewhat unique, in that potentially multiple routes of exposure to Hg exist, and exposure to multiple forms of Hg can occur. Hg vapors are released to the environment during extraction of gold using $Hg^0$-based amalgams and $Hg^0$ waste is subsequently disposed into water sources, where it is converted into MeHg. Gold miners are not only exposed to $Hg^0$ dermally and through vapors, but also to MeHg because of their common dietary practices of fish consumption (Ashe, 2012; Fraser, 2016; Gardner, 2012; Wade, 2013). South American exposure to mercurials through mining has made it the current largest known source of Hg pollution in the world (Wade, 2013), and one of the most extensive known adult Hg and MeHg exposures. As many as 48,000 people are estimated to have been affected so far; neurological effects levels of exposure have not yet been reported (Fraser, 2016). In 2012, it was reported that approximately 11% of the population at Madre de Dios mining zones had hair-Hg concentrations exceeding 16 mg Hg/g dry hair, a symptomatic concentration as described by the World Health Organization (Ashe, 2012). Recent reports indicating if and how these levels have changed are lacking. However, based on the reports we discuss in this review, if exposure to MeHg continues in Madre de Dios, Peru in a similar manner as has been reported since 2012, it is expected that this population will present neurological signs identical to those observed in MD.

Neurologic signs of MD correlate with degeneration of susceptible neuronal populations, including cerebellar granule cells and somatosensory neurons (Al-saleem, 1976; Bakir et al., 1973; Eto, 1997; Eto et al., 2002; Takeuchi et al., 1962). Although the precise sequence of cellular events leading to MeHg-induced neurodegeneration remains elusive, dysregulation of intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) appears to be a critical and early-onset component. MeHg exposure *in vitro* leads to a time- and concentration-dependent increase in $[Ca^{2+}]_i$ in multiple types of primary, and native neurons as well as immortalized cells (Bradford et al., 2016; Edwards et al., 2005; Hare et al., 1993; Johnson et al., 2011; Marty and Atchison, 1997b; Ramanathan and Atchison, 2011; Yuan and Atchison, 2007).

MeHg-induced alterations in $[Ca^{2+}]_i$ can be measured using single-cell $Ca^{2+}$ microfluorimetry. Cells in culture are loaded with the fluorophore fura-2-acetoxymethyl ester which readily crosses the cell membrane, after which it is deesterified by endogenous esterases and capable of binding free $Ca^{2+}$. Changes in $[Ca^{2+}]_i$ are then measured at 505 nm by calculating the ratio of fluorescence obtained using alternating excitation at 340 nm and 380 nm, corresponding to fura-2 bound and unbound to $Ca^{2+}{}_i$, respectively (Grynkiewicz et al., 1985; Limke and Atchison, 2009). MeHg increases $[Ca^{2+}]_i$ in two kinetically-distinct phases: the first (referred to as Phase 1) results from release of $Ca^{2+}$ from intracellular stores, whereas the second (Phase 2) corresponds with the influx of extracellular $Ca^{2+}$ in primary neurons in culture (Edwards et al., 2005; Limke et al., 2004a,b; Marty and Atchison, 1997, 1998; Ramanathan and Atchison, 2011), transformed cells (Hare et al., 1993; Hare and Atchison, 1995a,b), and using recombinant channels in heterologous expression systems (unpublished observation). For brain slices such as those of cerebellum, confocal microscopy is used in place of single cell fluorimetry (Bradford et al., 2016; Johnson et al., 2011; Yuan and Atchison, 2007), because confocal lasers cannot attain the spectral levels needed for excitation of fura-2. Using confocal microscopy, it is impossible to identify distinct phases of elevation of $Ca^{2+}{}_i$ in these preparations. On the other hand, confocal microscopy allows examination of comparative spatial sensitivity of distinct neuronal populations to MeHg-induced $[Ca^{2+}]_i$ dysregulation. For example, Yuan and Atchison (2009) showed a clear differential sensitivity to MeHg-induced changes in fluo-4 fluorescence between cerebellar granule and Purkinje cells in freshly prepared cerebellar slices. Moreover, in cerebellar organotypic slice culture, elevations of $[Ca^{2+}]_i$ are also observed in response to low concentrations of MeHg (Bradford et al., 2016), in a developmental migratory stage-dependent manner in granule neurons. Thus MeHg-induced elevations of $[Ca^{2+}]_i$ occur in both isolated cells, and intact circuits.

Consistent results also occur at the whole animal level, or following *in vivo* dosing. Chronic exposure to MeHg in drinking water, at concentrations which do not of themselves cause overt neurotoxic effects, causes elevations of $[Ca^{2+}]_i$ in brainstem slices of mice harboring a mutation in superoxide dismutase-1 (SOD1-G93A) (Johnson et al., 2011). Moreover, in rats treated with MeHg (5 mg/kg/day, p.o., 12 days), and concomitantly with L-type ($Ca_v1.3$) $Ca^{2+}$ channel blockers, there was a reduced incidence of gross signs of MeHg toxicity, compared with treats treated with MeHg alone (Sakamoto et al., 1996). Finally, in mice, dietary supplementation with the L-type $Ca^{2+}$ channel antagonist nimodipine, delayed, or precluded MeHg-induced behavioral toxicity (Bailey et al., 2013). Taken together, results from isolated cells, brain slices, and *in vivo* exposures provide compelling evidence for the pivotal role of elevations in intracellular $Ca^{2+}$ concentration. Additional effects, including generation of reactive oxygen species, and mitochondrial damage, occur in neurons following MeHg exposure. However, two studies have shown that, at least for cerebellar granule cells in culture, or synaptosomes derived from striatum, elevation of $[Ca^{2+}]_i$ is the *sine qua non* in MeHg-induced cytotoxicity (Dreiem and Seegal, 2007; Sarafian and Verity, 1991).