Available online at www.sciencedirect.com
SCIENCE DIRECT
Toxicology and Applied Pharmacology 208 (2005) 222–232
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Comparative sensitivity of rat cerebellar neurons to dysregulation of divalent cation homeostasis and cytotoxicity caused by methylmercury☆

Joshua R. Edwards, M. Sue Marty[1], William D. Atchison*

Department of Pharmacology and Toxicology, Michigan State University, B331 Life Sciences Building, East Lansing, MI 48824-1317, USA

Received 27 October 2004; accepted 5 February 2005
Available online 9 April 2005

Abstract

The objective of the present study was to determine the relative effectiveness of methylmercury (MeHg) to alter divalent cation homeostasis and cause cell death in MeHg-resistant cerebellar Purkinje and MeHg-sensitive granule neurons. Application of 0.5–5 μM MeHg to Purkinje and granule cells grown in culture caused a concentration- and time-dependent biphasic increase in fura-2 fluorescence. At 0.5 and 1 μM MeHg, the elevations of fura-2 fluorescence induced by MeHg were biphasic in both cell types, but significantly delayed in Purkinje as compared to granule cells. Application of the heavy-metal chelator, TPEN, to Purkinje cells caused a precipitous decline in a proportion of the fura-2 fluorescence signal, indicating that MeHg causes release of Ca^{2+} and non-Ca^{2+} divalent cations. Purkinje cells were also more resistant than granule cells to the neurotoxic effects of MeHg. At 24.5 h after-application of 5 μM MeHg, 97.7% of Purkinje cells were viable. At 3 μM MeHg there was no detectable loss of Purkinje cell viability. In contrast, only 40.6% of cerebellar granule cells were alive 24.5 h after application of 3 μM MeHg. In conclusion, Purkinje neurons in primary cultures appear to be more resistant to MeHg-induced dysregulation of divalent cation homeostasis and subsequent cell death when compared to cerebellar granule cells. There is a significant component of non-Ca^{2+} divalent cation released by MeHg in Purkinje neurons.

Keywords: Calcium; Calcium-mediated cell death; Cerebellar granule neuron; Divalent cation homeostasis; Fura-2; Intracellular calcium regulation; Methylmercury; Purkinje neuron; TPEN

Introduction

Methylmercury (MeHg) is a potent environmental neurotoxicant that causes selective cell death in certain populations of neurons. In both animals and humans, postnatal exposure to MeHg causes preferential effects on cerebellar granule cells while the adjacent Purkinje cells apparently remain, at least initially, unharmed (Hunter and Russell, 1954; Leyshon and Morgan, 1991; Leyshon-Sorland et al., 1994; Mori et al., 2000). The cellular or molecular mechanisms which contribute to the differential neurotoxic effects of MeHg within the cerebellum are, as yet, unknown. Because Purkinje cells are one of the largest neurons in the central nervous system (CNS) and cerebellar granule cells are one of the smallest, Clarkson (1972) suggested that cell size may be a contributing factor in the differential neurotoxic effects of MeHg. Other factors have been suggested to explain why Purkinje cells are more resistant to the neurotoxic effects of MeHg including the ability to chelate metals (Leyshon-Sorland et al., 1994), synaptic transmission (Yuan and Atchison, 2003), glutamate receptor subtype expression, and production of nitric oxide (Himi et al.,

Abbreviations: Calbindin-D28k (CB); HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; MeHg, Methylmercury; TPEN, *N*,*N*,*N*′,*N*′-tetrakis (2-pyridylmethyl)ethylenediamine.

☆ Preliminary accounts of this work were presented at the 2001 Annual Meeting of the Society for Neuroscience and the 1998 Annual Meeting of the Society of Toxicology and published, respectively, in abstract form (Soc. Neurosci. Abstr. 27, 383.3, 2001) and (The Toxicologist 42 (1–5): 194, 1998).

* Corresponding author. Fax: +1 517 432 1341.
E-mail address: atchiso1@msu.edu (W.D. Atchison).
[1] Present address: Toxicology and Environmental Research and Consulting, The Dow Chemical Company, 1803 Building, Midland, MI 48674, USA.

0041-008X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.taap.2005.02.015

1996). Because MeHg disrupts a wide variety of cellular processes, it is difficult to pinpoint individual, specific factors that may contribute to the differential neurotoxicity of MeHg observed in cerebellar granule and Purkinje neuronal cell types.

Currently, there are no studies comparing directly the cellular effects of MeHg on cerebellar Purkinje and granule cells. Because MeHg has high affinity for −SH groups and −SH groups are ubiquitous within cells, MeHg disrupts or alters several cell processes such as astrocyte function (Aschner et al., 1998), myelin formation (Chang, 1977), neurotransmitter release (Atchison and Narahashi, 1982; Juang, 1976), and neurotransmitter re-uptake (Komulainen and Tuomisto, 1982; O'Kusky and McGeer, 1989). Experiments utilizing a variety of in vitro preparations have shown that one of the earliest effects of MeHg is disruption of homeostasis of Ca^{2+} as well as non-Ca^{2+} divalent cations (Denny et al., 1993; Hare et al., 1993; Komulainen and Bondy, 1987; Limke and Atchison, 2002; Marty and Atchison, 1997; Sarafian, 1993). These effects occur at MeHg concentrations in the low-micromolar range in a variety of cell types (Hare et al., 1993), but can occur at submicromolar concentrations of MeHg in cerebellar granule cells (Limke and Atchison, 2002; Limke et al., 2003, 2004a; Marty and Atchison, 1997). MeHg causes a biphasic increase in intracellular calcium concentration ($[Ca^{2+}]_i$). The initial increase is associated with release of Ca^{2+} from intracellular stores. This is followed by a second increase due to entry of extracellular Ca^{2+} (Ca_e^{2+}) (Hare and Atchison, 1995a,b; Limke and Atchison, 2002; Limke et al., 2004a; Marty and Atchison, 1997). Furthermore, elevations of $[Ca^{2+}]_i$ by MeHg appear to be linked to cytotoxicity in cerebellar granule neurons in primary culture. Pre-treatment of granule cells with a cell-permeable form of the Ca^{2+} chelator BAPTA attenuated the incidence of MeHg-induced cytotoxicity 3.5 h after MeHg exposure (Marty and Atchison, 1998). In cerebellar granule cells and the NG-108-15 cell line, the time-to-onset of MeHg-induced elevations of $[Ca^{2+}]_i$ is inversely related to MeHg concentration although the maximum effect is not concentration-dependent (Hare et al., 1993; Limke and Atchison, 2002; Marty and Atchison, 1997). The specific pathways which contribute to each phase of increased $[Ca^{2+}]_i$ induced by MeHg are not completely clear. However, contributions of mitochondria and smooth endoplasmic reticulum appear to be involved as is participation by membrane Ca^{2+} channels. Specific antagonists of voltage-gated Ca^{2+} channels, muscarinic receptors, and the mitochondrial transition pore significantly delayed the time-to-onset of MeHg-induced elevations of $[Ca^{2+}]_i$ as well as attenuated MeHg-induced cell death (Limke and Atchison, 2002; Limke et al., 2004a; Marty and Atchison, 1997, 1998).

Studies in whole animals also support the idea that elevated $[Ca^{2+}]_i$ has a role in MeHg-mediated cell death. Mori et al. (2000) used a Ca^{2+}-sensitive dye, alizarin red S, to detect Ca^{2+} deposits in cerebellar slices of rats that were treated by injection with dimethylmercury. As early as 7 days after exposure to dimethylmercury, cerebellar granule cells started to show accumulations of Ca^{2+}, which increased after further exposure to dimethylmercury. Gross atrophy of the granule cell layer was evident at later time points. Adjacent Purkinje cells did not show any sign of cell loss or death, nor did Purkinje cells accumulate Ca_i^{2+} as indicated by a lack of staining for Ca^{2+} deposits (Mori et al., 2000). Thus, the presence or absence of Ca^{2+} deposits within the cerebellum is directly correlated with sensitivity or resistance to MeHg neurotoxicity, respectively. Based on these in vitro and in vivo observations, alterations in $[Ca^{2+}]_i$ appear to be a contributory factor to MeHg-induced neurotoxicity.

Regulation of other divalent cations, besides Ca^{2+}, may also be disrupted during MeHg exposure (for a review, see Denny and Atchison, 1996; Limke et al., 2004b). One of the non-Ca^{2+} divalent cations affected by MeHg has been identified as Zn^{2+}. Using ^{19}F-BAPTA NMR, release of Zn^{2+} was detected from the soluble fraction of rat brain synaptosomes after exposure to MeHg (Denny and Atchison, 1994). Zn^{2+} modulates the function of many cell membrane receptor proteins including glutamate, GABA, and glycine as well as voltage-gated Ca^{2+} channels (for a review, see Choi and Koh, 1998). Zn^{2+} can itself be cytotoxic. Cerebellar granule cells exposed to 100–500 μM Zn^{2+} for 15–30 min undergo concentration-dependent cell death (Manev et al., 1997). Thus, release of Zn_i^{2+} could contribute to cytotoxicity of granule cells in response to MeHg.

The purpose of this study was to compare the ability of MeHg to disrupt divalent cation homeostasis, as measured by changes in fura-2 fluorescence, and induce cytotoxicity in Purkinje and cerebellar granule neurons. The heavy-metal chelator, TPEN, was used to assess the effectiveness of MeHg to disrupt non-Ca^{2+} divalent cation homeostasis in Purkinje neurons.

Methods

Materials. Methylmercuric chloride (MeHg) was purchased from ICN Biochemicals Inc. (Aurora, OH) (95% pure). Tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN), fura-2 acetoxymethyl ester (fura-2 AM), and pluronic acid were purchased from Molecular Probes Inc. (Eugene, OR). 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), poly-L-ornithine (P-4638), anti-calbindin-D28k (C-9848), cytosine-arabinofuranoside (Ara-C) (C-1768), and sodium selenite (S-1382) were purchased from Sigma (St. Louis, MO). Goat anti-mouse IgG conjugated to tetramethyl rhodamine (TRITC) (715-025-150) was purchased from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA). Trypsin (LS003707) and deoxyribonuclease (LS002139) were purchased from Worthington Biochemical Corp. (Freehold, NJ). Fetal bovine serum (FBS) and Dulbecco's