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SCIENCE DIRECT

Molecular Brain Research 137 (2005) 11 – 22

MOLECULAR BRAIN RESEARCH

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Research report

Modulatory effect of glutathione status and antioxidants on methylmercury-induced free radical formation in primary cultures of cerebral astrocytes

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Accepted 5 February 2005
Available online 17 March 2005

Abstract

Excessive free radical formation has been implicated as one of the causative factors in neurotoxic damage associated with variety of metals, including methylmercury (MeHg). Although the mechanism(s) associated with MeHg-dependent neurotoxicity remains far from clear, overwhelming data give credence to a mediatory role for astrocytes, a major cell type that preferentially accumulates MeHg. To extend our recent findings of MeHg-induced increase in ROS formation (G. Shanker, J.L. Aschner, T. Syversen et al., Free radical formation in cerebral cortical astrocytes in culture induced by methylmercury, Mol. Brain Res. 128 (2004) 48–57), the present studies were designed to assess the effect of modulating intracellular glutathione (GSH) content, on ROS generation, in the absence and presence of MeHg. Intracellular GSH was reduced by treatment with 100 μM buthionine-L-sulfoxane (BSO) for 24 h, and increased by treatment with 1 mM L-2-oxothiazolidine-4-carboxylic acid (OTC) for 24 h. Additionally, the effects of the selective antioxidants, catalase (1000 U/ml for 1 h), an H_2O_2 scavenger, and *n*-propyl gallate (100 μM for 1 h), a superoxide radical ($^{\bullet}O_2^-$) and possibly hydroxyl radical ($^{\bullet}OH$) scavenger on MeHg-induced ROS formation were examined. After these treatments, astrocytes were exposed to ±10 μM MeHg for 30 min, following which the fluorescent probes, CM-H_2DCFA and CM-H_2XRos were added; 20 min later, laser scanning confocal microscopy (LSCM) images were obtained. Exposure of astrocytes for 24 h to 100 μM BSO, a GSH synthesis inhibitor, led to a significant increase in mitochondrial ROS (i.e., $^{\bullet}O_2^-$, $^{\bullet}NO$, and $ONOO^-$) formation, as assessed with CM-H_2XRos mitotracker red dye. Similarly, BSO increased ROS formation in various intracellular organelles, as assessed with CM-H_2DCFDA. BSO in combination with MeHg increased fluorescence levels in astrocytes to levels above those noted with BSO or MeHg alone, but this effect was statistically indistinguishable from either of these groups (BSO or MeHg). Pretreatment of astrocytes for 24 h with 1 mM OTC abolished the MeHg-induced increase in ROS. Results similar to those obtained with OTC were observed with the free radical scavenger, *n*-propyl gallate (n-PG). The latter had no significant effects on astrocytic fluorescence when administered alone. This $^{\bullet}O_2^-$ and possibly $^{\bullet}OH$ radical scavenger significantly attenuated MeHg-induced ROS formation. Catalase, an H_2O_2 scavenger, was less effective in reducing MeHg-induced ROS formation. Taken together, these studies point to the important protective effect of adequate intracellular GSH content as well as antioxidants against MeHg-triggered oxidative stress in primary astrocyte cultures.

Theme: Disorders of the nervous system
Topic: Neurotoxicology

Keywords: Astrocytes; Methylmercury; Neurotoxicity; Laser scanning confocal microscopy; Reactive oxygen species; Glutathione; Redox status; Mitochondria; Catalase; *n*-Propyl gallate

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doi:10.1016/j.molbrainres.2005.02.006

1. Introduction

Methylmercury (MeHg) is a significant environmental contaminant with well-known risks to human health. Excessive MeHg ingestion from a diet rich in fish has been linked to aberrant central nervous system (CNS) function. This ubiquitous environmental contaminant is capable of causing toxic effects as indicated by human poisoning epidemics following food-borne MeHg ingestion [11,65]. MeHg can easily pass placental and blood–brain barriers and cause CNS damage to both adult and developing brain [24,43]. Maternal mercury ingestion during pregnancy causes neurological as well as neuropsychological deficits in the offspring [25,34]. Another recent finding points to the damaging effects of MeHg on neurogenesis [29]. However, other studies found no definite connection between MeHg and neurodevelopmental deficits in children at 66 months of age [27,49]. Thus, there remains a clear need for additional studies on the basic mechanisms and consequences of MeHg exposure on brain function.

Numerous studies have established a prominent role for astrocytes in mediating MeHg neurotoxicity [22,33]. Astrocytes are a preferential cellular site for MeHg accumulation [6,21,31], MeHg induces inhibition of glutamate, cystine and cysteine uptake, thus adversely affecting intracellular GSH content and redox status in astrocytes [3,4,18,26,54,56,58], and MeHg also stimulates cytosolic phospholipase A_2 ($cPLA_2$) leading to arachidonic acid (AA) release from astrocytes, further inhibiting glutamate transporters [7,57].

Oxidative stress has been associated with a wide variety of neurodegenerative states as well as with metal-induced neurotoxicity [5,19,40,44,46]. In vivo as well as in vitro biochemical investigations using neuronal cultures, mixed neuronal/glial cultures, and recent studies with primary astrocytic cultures have demonstrated enhanced ROS formation with MeHg exposure [1,32,48,51–53,55,59,62,67].

One of the most effective and powerful techniques to examine oxidative stress is LSCM. With this technique, the approach to intracellular ROS detection is based on alterations in the fluorescence intensity of redox-sensitive

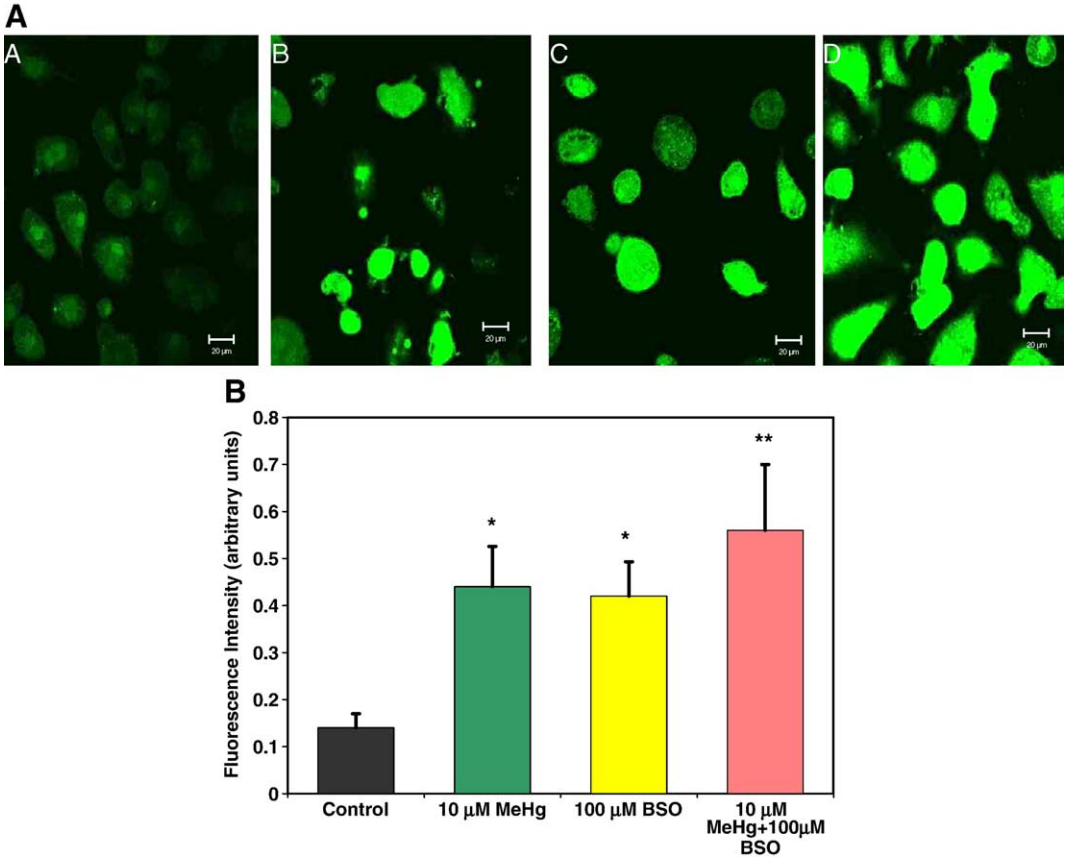

Fig. 1. (A) ROS production in rat primary cerebral astrocytes exposed to MeHg and BSO, assessed by changes in DCF fluorescence. Some of the culture dishes were pretreated for 24 h at 37 °C with 100 μM BSO, after which ±10 μM MeHg was added for an additional 30 min. Twenty minutes after the addition of CM-H_2DCFDA (7 μM), fluorescent images were recorded by LSCM. The images demonstrate an enhancement of fluorescent intensity with MeHg, BSO, as well as with the combination of BSO and MeHg (the images shown are representative of three separate studies conducted in three different astrocytes cultures, A = Control; B = 10 μM MeHg; C = 100 μM BSO; D = 100 μM BSO plus 10 μM MeHg). Scale bar = 20 μm. (B) The images obtained in panel A were quantitatively analyzed for changes in fluorescence intensities within cells using the Zeiss LSM software. Data were collected from 3 different control and treated cultures (total number of cells analyzed = 12–18). The results indicate a significant increase in fluorescent intensity with both MeHg and BSO alone as well as in combination (*$P < 0.05$ versus control; **$P < 0.01$ versus control; mean ± SEM).