Available online at www.sciencedirect.com
ScienceDirect
NeuroToxicology 27 (2006) 970–981
NeuroToxicology

Methylmercury elicits rapid inhibition of cell proliferation in the developing brain and decreases cell cycle regulator, cyclin E

Kelly Burke [a], Yinghong Cheng [a], Baogang Li [a], Alex Petrov [a], Pushkar Joshi [a], Robert F. Berman [b], Kenneth R. Reuhl [c], Emanuel DiCicco-Bloom [a,d,e,*]

[a] Department of Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, United States
[b] Department of Neurological Surgery, University of California at Davis, United States
[c] Department of Pharmacology & Toxicology, Rutgers University, United States
[d] Department of Pediatrics, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, United States
[e] Cancer Institute of New Jersey, New Brunswick, NJ, United States

Received 28 March 2006; accepted 8 September 2006
Available online 15 September 2006

Abstract

The developing brain is highly sensitive to methylmercury (MeHg). Still, the initial changes in cell proliferation that may contribute to long-term MeHg effects are largely undefined. Our previous studies with growth factors indicate that acute alterations of the G1/S-phase transition can permanently affect cell numbers and organ size. Therefore, we determined whether an environmental toxicant could also impact brain development with rapid (6–7 h) effects on DNA synthesis and cell cycle machinery in neuronal precursors. *In vivo* studies in newborn rat hippocampus and cerebellum, two regions of postnatal neurogenesis, were followed by *in vitro* analysis of two precursor models, cortical and cerebellar cells, focusing on the proteins that regulate the G1/S transition. In postnatal day 7 (P7) pups, a single subcutaneous injection of MeHg (3 μg/g) acutely (7 h) decreased DNA synthesis in the hippocampus by 40% and produced long-term (2 weeks) reductions in total cell number, estimated by DNA quantification. Surprisingly, cerebellar granule cells were resistant to MeHg effects *in vivo* at comparable tissue concentrations, suggesting region-specific differences in precursor populations. *In vitro*, MeHg altered proliferation and cell viability, with DNA synthesis selectively inhibited at an early timepoint (6 h) corresponding to our *in vivo* observations. Considering that G1/S regulators are targets of exogenous signals, we used a well-defined cortical cell model to examine MeHg effects on relevant cyclin-dependent kinases (CDK) and CDK inhibitors. At 6 h, MeHg decreased by 75% levels of cyclin E, a cell cycle regulator with roles in proliferation and apoptosis, without altering p57, p27, or CDK2 nor levels of activated caspase 3. In aggregate, our observations identify the G1/S transition as an early target of MeHg toxicity and raise the possibility that cyclin E degradation contributes to both decreased proliferation and eventual cell death.

Keywords: Mercury; Neurogenesis; Neural stem cell; Cell cycle; Cyclin E; Cell survival; Cerebral cortex; Cerebellar granule precursors; Hippocampus; Proliferation

1. Introduction

Methylmercury (MeHg) exposure produces widespread abnormalities in the developing brain, including reduced organ size and altered neuronal migration. *In utero* exposure leads to clinical deficits at exposure levels not associated with maternal symptoms (Chang et al., 1977; Choi et al., 1978; Choi, 1989). Considering the distinct sensitivity of the fetus, recent epidemiological studies have investigated the possible developmental effects of early exposure to dietary levels of MeHg (Davidson et al., 1998; Grandjean et al., 1997; Myers et al., 2003). While these studies have produced ambiguous, often contradictory, results (Spurgeon, 2006), animal studies reveal behavioral deficits at MeHg exposures that do not produce histologic changes (Sakamoto et al., 2004), implying that subtle MeHg effects may occur at subtoxic doses.

Mercury damages cells in multiple ways, by generating oxidative stress, binding critical sulfhydryl groups, depolymer-

* Corresponding author at: Department of Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Medical School, 675 Hoes Lane, Room RWJSPH 362, Piscataway, NJ 08854, United States. Tel.: +1 723 235 5388.
E-mail address: diciccem@umdnj.edu (E. DiCicco-Bloom).

0161-813X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2006.09.001

izing microtubules, and altering neurotransmission (Castoldi et al., 2001). Apoptosis or necrosis ensues depending on the level of exposure (Kuo and Lin-Shiau, 2004). In proliferating neuronal precursors of the cerebellum, MeHg impedes the production of new neurons by disassembling spindle microtubules and producing metaphase arrest (Rodier et al., 1984). MeHg effects at earlier phases of the cell cycle have been identified but are less well defined (Faustman et al., 2002; Roy et al., 1991). In addition, many MeHg studies on cellular toxicity (Castoldi et al., 2001), neurogenesis (Faustman et al., 2002), and long-term effects (Goulet et al., 2003; Rossi et al., 1997; Sakamoto et al., 2002) have used chronic dosing or timepoints $\geq$24 h. Few studies have investigated early, single-dose effects on neurogenesis.

Neurogenesis refers to the production of new cells, both neurons and glia, in the developing brain. Extracellular signals can alter this process by stimulating or inhibiting cell cycle progression from G1 into DNA synthetic S-phase. Under favorable growth conditions, mitogen-dependent cyclins interact with cyclin-dependent kinases (CDKs) to promote entry into S-phase and facilitate cell proliferation. Conversely, endogenous anti-mitogenic signals act via CDK inhibitors, such as p27 and p57, to reduce cyclin-CDK complex activity and prevent G1/S transition (Cunningham and Roussel, 2001; Sherr and Roberts, 1999). Previous studies have identified positive and negative regulators of G1/S transition that acutely (8 h) enhance or restrict cell proliferation, respectively (Carey et al., 2002; Li and DiCicco-Bloom, 2004; Wagner et al., 1999). Whether neurotoxicants can act in similar fashion is unknown.

Cell cycle regulation influences the number and position of neurons in the cerebral cortex (Levitt et al., 1997; Takahashi et al., 1995, 1999; Vaccarino et al., 1999), hippocampus (Altman and Bayer, 1990), and cerebellum (Hatten and Heintz, 1995). Within these structures, there are spatially distinct regions of proliferating cells that generate neurons at specific developmental periods: the forebrain ventricular zone produces neurons in the embryonic cerebral cortex, while granule neurons are produced postnatally in the hippocampal dentate gyrus and the external germinal layer (EGL) of the cerebellum (reviewed in DiCicco-Bloom and Sondell (2005)). Acute mitogenic regulation of precursor populations can have long-term effects. In postnatal hippocampus and cerebellum, enhanced neurogenesis follows basic fibroblast growth factor (bFGF) administration to newborn rats and produces sustained growth in both regions (Cheng et al., 2001, 2002). Interestingly, these neurogenetic effects do not extend to adult hippocampus, suggesting that developing neurons are uniquely susceptible to neurogenetic signals (Wagner et al., 1999), which may include environmental toxicants.

Based on these observations, MeHg could interact with cell cycle machinery to acutely disrupt proliferation and consequent cell numbers, leading to subtle effects during development. To investigate this possibility, we followed the bFGF model used in previous studies *in vivo*, and found that MeHg decreases DNA synthesis acutely in hippocampus but not cerebellum (Burke et al., 2004) and leads to long-term changes in cell number. We used a single subcutaneous injection of MeHg because it allowed highly reproducible control of mercury levels as well as precise temporal analyses of cell cycle and cell death pathways. We then focused on cell cycle regulators, using well-defined culture models to assess changes in DNA synthesis, cell survival, G1/S transition, and CDK levels. While changes in cell viability paralleled reductions in DNA synthesis at 24 h, earlier reductions at 6 h occurred independently of cell death. Furthermore, effects at 6 h were associated with a selective decrease in cyclin E, a molecule crucial for S-phase entry (Sherr and Roberts, 1999) but also involved in apoptosis (Mazumder et al., 2002). Our data support an additional cell cycle locus, namely the G1/S transition, for MeHg toxicity and raise the possibility that these rapid, region-specific effects depend on altered cell cycle machinery and lead to long-term consequences on regional brain development.

2. Materials and methods

2.1. Materials

Methylmercury chloride (CH_3HgCl) was purchased from Spectrum (Gardena, CA) or MP Biomedicals (Irvine, CA). A 1 mg/ml stock solution in 0.1 M phosphate buffered saline (PBS) was prepared immediately before use and dissolved by sonication. Dilutions were made in PBS for injections or in defined media for addition to cell culture.

2.2. Animal treatment

Time-mated Sprague–Dawley rats were obtained from Hilltop Lab Animals, Inc. (Philadelphia, PA) with plug date designated as embryonic day 0 (E0) or litter birth date as postnatal day 0 (P0). For *in vivo* studies, P7 rats were injected subcutaneously (sc) with vehicle or MeHg (0.1–30 μg/g) in a 50–100 μl bolus. Animals were sacrificed by decapitation at 7 h, 24 h, and in some cases, at 2 weeks. The hippocampus and cerebellum were removed from whole brains, cleaned of meninges, and frozen at −80 °C until processing. Cell cultures were obtained from P7 pups (for granule cells) or E14.5 embryos (for cortical precursors). Pregnant females were sacrificed by CO_2 inhalation and embryos removed immediately. All animal procedures were approved by the Robert Wood Johnson Medical School institutional animal care and utilization committee and conformed to NIH Guidelines for animal use.

2.3. Cortical precursor culture

To obtain a homogeneous population, dorsolateral cerebral cortex was separated from basal ganglia, hippocampus, and overlying meninges. Cells were mechanically dissociated, plated on 0.1 mg/ml poly-D-lysine coated culture dishes, and incubated at 37 °C with 5% CO_2 in defined media (Lu and DiCicco-Bloom, 1997) composed of DMEM and F12 (50:50 (v/v); Invitrogen, Grand Island, NY) and containing penicillin (50 U/ml), streptomycin (50 μg/ml), transferrin (100 μg/ml) (Calbiochem, La Jolla, CA), putrescine (100 μM), progesterone (20 nM), selenium (30 nM), glutamine (2 mM), glucose (6 mg/ml), and