Comparative Biochemistry and Physiology, Part C 157 (2013) 322–327

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Effects of methylmercury on epigenetic markers in three model species: Mink, chicken and yellow perch

Niladri Basu [a,*], Jessica Head [a,b], Dong-Ha Nam [a,c], J. Richard Pilsner [d], Michael J. Carvan [e], Hing Man Chan [f], Frederick W. Goetz [g], Cheryl A. Murphy [h], Kirsti Rouvinen-Watt [i], Anton M. Scheuhammer [j]

[a] Department of Environmental Health Sciences, University of Michigan, 1415 Washington Heights, Ann Arbor, MI 48109, USA
[b] Cooperative Institute for Limnology and Ecosystem Research, University of Michigan, 440 Church Street, Ann Arbor, MI 48109, USA
[c] Department of Biological Sciences, College of Natural Sciences, Chonnam National University, 77 Yongbong-ro Bukgu, Gwangju, 500-757, South Korea
[d] Division of Environmental Health Sciences, University of Massachusetts—Amherst, 686 N. Pleasant Street, Amherst, MA 01003, USA
[e] Great Lakes Wisconsin Aquaculture Technology and Environmental Research (WATER) Institute, University of Wisconsin—Milwaukee, 600 E. Greenfield Ave, Milwaukee, WI 53204, USA
[f] Center for Advanced Research in Environmental Genomics (CAREG), University of Ottawa, 30 Marie Curie, Ottawa, ON, Canada K1N 6N5
[g] Northwest Fisheries Science Center, NOAA, 2725 Montlake Blvd East, Seattle, WA 98112, USA
[h] Department of Fisheries and Wildlife, Michigan State University, 480 Wilson Rd, Lansing, MI 48824, USA
[i] Faculty of Agriculture, Dalhousie University, PO Box 550, Truro, NS, Canada B2N 5E3
[j] National Wildlife Research Center, Environment Canada, Ottawa, ON, Canada K1A 0H3

ARTICLE INFO

Article history:
Received 31 October 2012
Received in revised form 17 February 2013
Accepted 19 February 2013
Available online 26 February 2013

Keywords:
Mercury
Ecotoxicology
Wildlife
Environmental epigenetics
DNA methylation

ABSTRACT

We previously reported that methylmercury (MeHg) exposure is associated with DNA hypomethylation in the brain stem of male polar bears. Here, we conveniently use archived tissues obtained from controlled laboratory exposure studies to look for evidence that MeHg can disrupt DNA methylation across taxa. Brain (cerebrum) tissues from MeHg-exposed mink (*Neovison vison*), chicken (*Gallus gallus*) and yellow perch (*Perca flavescens*) were analyzed for total Hg levels and global DNA methylation. Tissues from chicken and mink, but not perch, were also analyzed for DNA methyltransferase (DNMT) activity. In mink we observed significant reductions in global DNA methylation in an environmentally-relevant dietary exposure group (1 ppm MeHg), but not in a higher group (2 ppm MeHg). DNMT activity was significantly reduced in all treatment groups. In chicken or yellow perch, no statistically significant effects of MeHg were observed. Dose-dependent trends were observed in the chicken data but the direction of the change was not consistent between the two endpoints. Our results suggest that MeHg can be epigenetically active in that it has the capacity to affect DNA methylation in mammals. The variability in results across species may suggest inter-taxa differences in epigenetic responses to MeHg, or may be related to differences among the exposure scenarios used as animals were exposed to MeHg through different routes (dietary, egg injection), for different periods of time (19–89 days) and at different life stages (embryonic, juvenile, adult).

1. Introduction

Methylmercury (MeHg) is a ubiquitous toxicant that biomagnifies through aquatic food webs (Scheuhammer et al., 2007). Once inside the body, MeHg is effectively absorbed from the bloodstream and can readily enter sensitive tissues such as the brain (Clarkson and Magos, 2006). Numerous studies have documented that under real-world exposure scenarios, MeHg causes adverse effects to the health of individuals and populations of various taxa, including fish (Depew et al., 2012a), birds (Depew et al., 2012b), and mammals (Basu and Head, 2010).

The toxic actions of MeHg are diverse owing to its high affinity for thiol groups (Clarkson and Magos, 2006). A myriad of effects have been described at a molecular and cellular level including changes in neurotransmission, oxidative stress, and gene expression. There is now preliminary evidence that MeHg, like other toxic metals, may cause epigenetic changes (Pilsner et al., 2010). Epigenetics refers to heritable factors affecting gene expression that occur outside of modifications to the DNA sequence itself (Robertson and Wolffe, 2000; Head et al., 2012). These include DNA methylation and histone modification. Epigenetic marks are susceptible to environmental influences such as exposure to chemicals, and these changes may be inherited as cells divide mitotically, or even between generations. Epigenetic mechanisms may even help explain the observation of temporal disconnects between exposure and effect, as early-life exposure may leave epigenetic marks that result in adverse health outcomes later in life. This is noteworthy as MeHg is known to have a

* Corresponding author at: Department of Environmental Health Sciences, 109 S. Observatory Street, University of Michigan, Ann Arbor, Michigan, 48109-2029, USA. Tel.: +1 734 764 9490; fax: +1 734 936 7283.
E-mail address: niladri@umich.edu (N. Basu).

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http://dx.doi.org/10.1016/j.cbpc.2013.02.004

long latency of effect in many organisms (Clarkson and Magos, 2006; Basu and Head, 2010).

In the biomedical sciences, MeHg-associated changes in epigenetic markers are starting to be found in several experimental model systems. Developmental exposure of mice to MeHg resulted in a range of epigenetic changes in brain-derived neurotrophic factor (BDNF) in male offspring, including increased DNA methylation and altered methylation and acetylation of histones (Onishchenko et al., 2008). These epigenetic changes were associated with depressive symptoms. In female offspring of rats exposed to MeHg throughout pregnancy, reduced hepatic expression of DNA methyltransferases (DNMT1, DNMT3a, DNMT3b) was reported (Desaulniers et al., 2009). DNA methyltransferases are a family of enzymes that establish and maintain patterns of DNA methylation in the genome. An in vitro study using mouse embryonic cells found that acute exposure to Hg^{2+} impairs histone production and reduces H3-K27 methylation (Gadhia et al., 2012). Such epigenetic findings have been extended to humans as increased methylation of GSTM1/5 promoter in blood was associated with MeHg exposure in women undergoing in vitro fertilization (Hanna et al., 2012), and hypomethylation of *SEPP1* was associated with hair Hg levels among a cohort of male dentists (Goodrich et al., in press). Collectively these aforementioned studies provide founding evidence that mercury compounds can cause epigenetic changes.

Of all organisms, fish-eating wildlife are among those with the greatest exposures to MeHg (Scheuhammer et al., 2007; Basu and Head, 2010). However, little is known about MeHg-associated epigenetic effects in these organisms, (Vandegehuchte and Janssen, 2011; Head et al., 2012). We previously observed reduced global DNA methylation in association with MeHg exposure in brain stem tissues of male polar bears (Pilsner et al., 2010). While the results of this polar bear study were informative, the DNA methylation results were highly variable and likely influenced by a range of known (e.g., other toxicants, health status, age, gender, tissue quality) and unknown factors that could not be well-controlled when studying tissues from wild-caught animals. Given the need to generate data from well-controlled laboratory experiments, the current study was performed to increase understanding of possible MeHg-associated epigenetic changes using a mammalian, avian, and fish model species. All three classes contain species with well-documented sensitivities to MeHg (Scheuhammer et al., 2007). For each class we chose to study a model ecotoxicological test organism, namely mink (*Neovison vison*), chicken (*Gallus gallus domesticus*), and yellow perch (*Perca flavescens*). As part of other research projects, animals of each species were previously exposed in the laboratory to levels of MeHg ranging from ecologically relevant to high. Here we opportunistically used tissues preserved from those studies. The brain tissue (focus: cerebrum) was investigated in each model since this organ is the principal target of MeHg (Clarkson and Magos, 2006; Basu and Head, 2010). We examined global genomic DNA methylation using the LUMA assay and the enzymatic activity of DNMT via a commercially available ELISA kit. The guiding hypothesis of this study was that brain global genomic DNA methylation and DNMT enzyme activity would be reduced in association with methylmercury exposure in each of the three model species.

2. Materials and methods

2.1. Animal exposure studies

Mink have been exposed to a range of contaminants in the laboratory largely for the purposes of ecological risk assessment, and in the field they have been shown to accumulate potentially toxic concentrations of MeHg (Basu et al., 2007a). Here, captive juvenile male mink ($n = 12$ per treatment) were exposed daily to MeHgCl (0, 0.1, 0.5, 1, and 2 mg/kg nominal concentrations in diet) as previously described (Basu et al., 2006, 2007b). Following 3 months of exposure, animals were euthanized, occipital cortex dissected, immediately frozen on dry ice and stored at −80 °C. All aspects of this study were approved institutionally by the animal ethics committees of McGill University and the Nova Scotia Agricultural College.

Avian toxicologists have routinely injected chicken eggs with contaminants to address a range of toxicological questions relating to risk assessment in wild birds (Heinz et al., 2009). Here, fertilized white leghorn chicken eggs were obtained from the Michigan State University Poultry Unit. Once received at the University of Michigan School of Public Health, they were air-cell injected with MeHgCl (non-inject, vehicle inject, 0.62, 2.0, 3.2, and 6.4 μg/g egg injected, nominal concentrations; $n = 9$ per treatment) on day 0 of incubation. Eggs were incubated at 37.7 °C under 55–60% humidity. On day 19 of embryonic development, embryos were euthanized by decapitation, and the cerebrum regions were dissected from brain and immediately frozen on dry ice and stored at −80 °C until analysis. All aspects of this study were approved by the University of Michigan Committee on Use and Care of Animals.

Perch are known to be sensitive to MeHg in nature (Wiener et al., 2012) and increasing number of studies are utilizing them as a laboratory model (Kwon et al., 2012). Here, female yellow perch were fed pellets containing nominal MeHgCl concentrations of 0, 0.5, 5, and 50 μg/g. Fish were fed twice daily for a period of 4 weeks. Fish were sacrificed and the telencephalon brain region was removed, immediately frozen on dry ice and stored at −80 °C until analyses. For each treatment there were three replicate tanks with five fish per tank, and for the purposes of this study we pooled together all five telencephalons from a given tank. For perch, we measured global genomic DNA methylation but not DNMT activity. All aspects of this study were approved by the University of Wisconsin—Milwaukee Animal Care and Use Committee.

2.2. Mercury analysis

Concentrations of total Hg were measured in the brain tissues of mink, chicken, and perch using methods described previously (Basu et al., 2006). For mink and chicken, individual brains were analyzed. For perch, pools of brain from five fish (all from a single tank) were analyzed. Total Hg content in each sample was measured using a Direct Mercury Analyzer 80 (Milestone Inc., Shelton, CT, USA). Analytical accuracy and precision were determined through the use of DOLT-3 standard reference material (National Research Council of Canada) and intermittent analysis of duplicate samples. Across the studies, average recovery (accuracy) was within 10% of expected and precision was less than 9%. Mercury concentrations are reported as μg/g (ppm) wet mass (ww), unless indicated.

2.3. DNA processing

Genomic DNA was isolated from all tissues using DNeasy kits (Qiagen) according to manufacturer's instructions. An optional RNAse treatment step was found to be essential for accurate fluorescence-based quantification of the isolated DNA. DNA concentration was measured using a nanodrop spectrophotometer (Thermo Fisher Scientific).

2.4. DNMT assay

Brain DNMT activity (EC 2.1.1.37) was assessed using a commercially available ELISA kit (EpiQuik™ DNMT Activity, Epigentek Group Inc., Farmingdale, NY, USA) in mink and chicken, but not in perch. Briefly, nuclear extracts were incubated for 1 h in wells coated with cytosine-rich DNA to enable DNMT-mediated methylation of substrate DNA. The methylated DNA was labeled with an anti-5-methylcytosine antibody, which was then detected colorimetrically following incubation with a secondary antibody. The absorbance values were blank-adjusted and the final DNMT activity was reported as absorbance (OD)/h/mg protein. Assays were performed in duplicate or triplicate and intra-plate variation (as RSD%) was less than 13.3%.