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Developmental selenomethionine and methylmercury exposures affect zebrafish learning

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ABSTRACT

Methylmercury (MeHg) is a ubiquitous environmental pollutant and has been shown to affect learning in vertebrates following relatively low exposures. Zebrafish were used to model long-term learning deficits after developmental MeHg exposure. Selenomethionine (SeMet) co-exposure was used to evaluate its role in neuroprotection. Embryos were exposed from 2 to 24 h post fertilization to (1) MeHg without SeMet, (2) SeMet without MeHg and (3) in combination of MeHg and SeMet. In case (1), the levels of MeHg were 0.00, 0.01, 0.03, 0.06, 0.10, and 0.30 μM. In case (2), the levels of SeMet were 0.00. 0.03, 0.06, 0.10, and 0.30 μM. In case (3), co-exposure levels of (MeHg, SeMet) were (0.03, 0.03), (0.03, 0.06), (0.03, 0.10), (0.03, 0.30), (0.10, 0.03), (0.10, 0.06), (0.10, 0.10), and (0.10, 0.30) μM. Learning functions were tested in individual adults, 4 months after developmental exposure using a spatial alternation paradigm with food delivery on alternating sides of the aquarium. Low levels of MeHg (<0.1 μM) exposure delayed learning in treated fish; fish exposed to higher MeHg levels were unable to learn the task; SeMet co-exposure did not prevent this deficit. These data are consistent with findings in laboratory rodents. The dorsal and lateral telencephalon are the primary brain regions in fish involved in spatial learning and memory. Adult telencephalon cell body density decreased significantly at all MeHg exposures >0.01 μM MeHg. SeMet co-exposure ameliorated but did not prevent changes in telencephalon cell body density. In summary, MeHg affected both learning and brain structure, but SeMet only partially reversed the latter.

1. Introduction

It has been documented in a number of mammalian species that methylmercury (MeHg) exposure can induce learning deficits [7,14,28,47–49,77]. However, a lack of unequivocal results from epidemiological studies on children [17,20,24] has lead researchers to question the direct association of MeHg exposure to behavioral deficits and potential neurotoxicological effects. The result of this controversy has been an examination of other components in the diet that may mitigate MeHg toxicity [21,54]. Recently, much discussion has focused on the interactions between Hg and selenium (Se), specifically selenoenzymes such as glutathione peroxidase and thyroid hormone deiodinases and their precursors [13,16,69,70]. It has been proposed that mercury (Hg), by binding to Se compounds, actually creates Se deficiencies that are the root cause of many of the toxic effects of Hg [69,70] and Se supplementation at moderate levels may provide neuroprotective effects against Hg toxicity [13,70]. At sufficiently high concentrations, Se, however, also can induce toxic effects [30,31].

Central to this study is the effect of Hg–Se interactions during embryo neurogenesis on adult behavior. It has been noted that developmental co-exposure to both selenomethionine (SeMet) and MeHg may result in differential effects in adult animals depending upon the specific behavior being evaluated, e.g., reduction in the severity of neurobehavioral deficits are observed only for simple reflex behaviors [71] but not for more complex learning tasks [59]. This study utilizes another vertebrate model, zebrafish, to address this discrepancy in behavioral outcomes due to developmental MeHg and SeMet co-exposure.

The fish dorsal and lateral telencephalon are critical for spatial learning of landmarks to identify foraging areas and nesting locations,

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and to navigate through their environments [23]. The size of these brain regions is correlated to the complexity of the individual's spatial environment as telencephalic ablations lead to decreased spatial learning [32,36,44,51,63]. Within the goldfish telencephalon, specific regions relate to specific forms of learning as indicated by ablation experiments; the lateral telencephalon relates to avoidance learning, whereas the medial telencephalon appears to be responsible for reversal spatial learning [56].

Using a variety of learning paradigms, e.g., conditioned aversion, conditioned reinforcement tasks, and prey capturing abilities, behavioral impairments in both rodents and fish have been identified after exposure to environmental contaminants, including MeHg [1,33,65,72,78]. While the effect of MeHg on spatial learning abilities in fish has not yet been examined, developmental exposures to lead (Pb^{2+}) or ethanol induced decreases in spatial learning abilities in zebrafish [11]. Since analyses of potential changes in telencephalon architecture or function were not recorded in that study, fundamental mechanisms of behavioral alteration could not be identified. This study utilizes zebrafish (*Danio rerio*) to assess some basic neurobehavioral alterations induced by developmental exposure to MeHg because of its utility as a vertebrate model system for examining the early neurogenesis of critical pathways that control specific, reproducible, easily observable behaviors [27,68].

2. Methods and results

2.1. Treatment of glassware and plasticware

All laboratory materials made of plastic were washed thoroughly in a 10% solution of a nontoxic, biodegradable detergent (Simple Green™; Sunshine Makers, Inc., Huntington Harbour, CA), rinsed repeatedly in ultrapure Milli-Q™ water (Millipore Corp., Medford, MA), and immersed in a 30 mM Na_4EDTA (Fisher Scientific, Hanover Park, IL) solution overnight to remove all surface adsorbed metal ions; glassware was washed and rinsed similarly but immersed in a 10% HNO_3 (Fisher Scientific, Hanover Park, IL) solution overnight. Glass and plasticware were then rinsed in ultrapure Milli-Q™ water.

2.2. Breeding and egg collection

Adult female (Tupfel long fin strain, Zebrafish International Resource Center, Eugene, OR) and male (golden leopard strain, Ekkwill Waterlife Resources, Gibsonton, FL) zebrafish were housed separately, and acclimated for several weeks prior to the initiation of experiments. Different strains were used to facilitate differentiation between sexes, as zebrafish do not show prominent signs of sexual dimorphism. Fish were maintained at 26–28 °C on a 14-hour light and 10-hour dark cycle in a flow-through buffered, de-chlorinated water system at the Aquatic Animal Facility of the University of Wisconsin—Milwaukee Children's Environmental Health Sciences Center. All experimental procedures were approved by the University of Wisconsin—Milwaukee Animal Care and Use Committee. Zebrafish were bred in 2-l plastic aquaria with a 1/8″ nylon mesh false bottom to protect fertilized eggs from being consumed by the adults. Eggs were collected ≤2 h post fertilization (hpf), counted, and placed into metal-free, glass culture dishes (100 mm diameter × 50 mm depth; $N = 100$ eggs/dish) in E2 medium [50] (each liter contains 0.875 g NaCl, 0.038 g KCl, 0.120 g $MgSO_4$, 0.021 g KH_2PO_4, and 0.006 g Na_2HPO_4).

2.3. Exposure regimen

Methylmercury (MeHg; >98% purity) was obtained from ICN Biomedicals (Aurora, OH). Seleno-L-methionine (SeMet; >98% purity) was obtained from Sigma Chemicals (St. Louis, MO). Collected eggs ($N = 100$ eggs/dish; <2 hpf) were rinsed twice in MeHg-free E2 medium (as determined by ICP-MS analysis) and transferred to metal-free glass dish (100 mm diameter × 50 mm depth) containing 100 ml of E2 medium with either only MeHg at 0.0, 0.01, 0.03, 0.06, 0.10, and 0.30 μM; or only SeMet at 0.0, 0.03, 0.06, 0.10, and 0.30 μM; the 0.0 μM MeHg and 0.0 μM SeMet sets were identical. These levels of developmental MeHg exposures were found to alter adult zebrafish visual startle responses; the above levels of SeMet co-exposures were found to reduce the behavioral response only at the highest concentrations [71]. In addition, developmental co-exposures (2–24 hpf) of 0.03 or 0.1 μM MeHg and 0.03, 0.06, 0.10, or 0.30 μM SeMet were also used, i.e., 8 separate combinations (refer to Table 3A for results of all combinations). These levels were found to mitigate MeHg-induced visual startle response alterations [71]. Higher concentrations were not used as 0.6 μM MeHg or SeMet were at or above the LC_{50}. At 24 hpf the embryos were rinsed in MeHg-free E2 medium and a subsample was analyzed for Hg and Se content by ICP-MS. The remainder were raised in MeHg-free E2 medium (28 °C; 14L:10D). Fry were fed vinegar eels twice each day starting at day 5 post-hatch regardless of treatment until large enough to consume *Artemia* nauplii. Juveniles and adults were fed Aquarian™ flake food (Aquarium Pharmaceuticals, Inc., Chalfont, PA) in the morning and *Artemia* nauplii in the afternoon. Based upon this and previous studies, there are no significant differences in embryo, larval, juvenile, or adult mortality or number of developmental malformations at the stated concentrations of either MeHg or SeMet. However, there are few data quantifying Se levels in these diets or if these potential sources of added Se have any effect on behavioral outcomes of MeHg-exposed fishes [67].

2.4. Embryo Hg and Se analysis

Metal analyses follow previously published protocols [71]. For each exposure concentration of MeHg and SeMet and co-exposure regimen of MeHg and SeMet the data were collected in 3 replicates. For each replicate, 100 eggs were collected after 24 hpf, rinsed twice in Hg-free E2 medium, placed in Teflon™ microvials (7.0 ml) with the chorion intact, and acid digested (2.0 ml of: 80 ml ICP-MS grade, ultrapure HNO_3 + 0.5 ml ICP-MS grade, ultrapure gold (Au) diluted to 1.0 l with Milli-Q™ water) in a microwave oven (MARS 5, CEM Corp., Matthews, NC). Gold was added to the digestion solution of both control and treated eggs to scavenge any Hg that might otherwise adsorb to the vessel wall and be unavailable for analysis. The "closed vessel" digestion was carried out under a temperature controlled program (25 °C to 130 °C at 5 °C per minute, held at 130 °C for 10 min, cooled to room temperature). Samples were decanted into 20 ml autosampler vials and brought to a final volume of 10.0 ml with the addition of 9.0 ml digestion solution. Mercury and selenium were measured with a MicroMass Platform inductively coupled plasma-mass spectrophotometer (ICP-MS) (Manchester, UK) equipped with a CETAC ASX 500 autosampler (Waters Corp, Medford, MA) under MassLynx NT software control for element measurements. Appropriate calibration standards were prepared from a 10 μg ml^{-1} (in 5% ICP-MS grade HNO_3) Hg or Se standard (CertiPrep, Metuchen, NJ). A calibration curve was constructed by ICP-MS analysis of 1–100 ppb Hg and Se. The solvent system solution blank was 5% HNO_3, 0.1% HCl and 500 ppb Au in ICP-MS grade, ultrapure water (18 MΩ). Acids used were of double-distilled ICP-MS grade (Optima, Fisher Scientific). All analyses were measured in the SIR Mode (Single Ion Recording) for 60 s. Separate analyses ($N = 3$/exposure) were conducted on the exposure media to verify concentrations. Data were recorded as medium MeHg or SeMet concentration vs. tissue residue (ppb) as the level of these substances in the body represents the effective exposure regimen for the developing embryo. Thus, control embryos may have residual levels of either mercury or selenium. The collected data are given in Tables 1A, 2A, and 3A. Since the specific speciation of either mercury or selenium was not determined, they are simply identified