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Dopaminergic dysregulation and impaired associative learning behavior in zebrafish during chronic dietary exposure to selenium☆

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ABSTRACT

A growing body of evidence indicates that exposure to selenium (Se) can cause neurotoxicity, and this can occur because of its interference with several neurotransmitter systems in humans and animals. Dopamine is a critical modulator of a variety of brain functions and a prime target for environmental neurotoxicants. However, effects of environmentally relevant concentrations of Se on dopaminergic system and its neurobehavioral effects are still largely unknown. For this purpose, we exposed zebrafish, a model organism, to different concentrations of dietary L-selenomethionine (control, 3.5, 11.1, 27.4, and 63.4 μg Se/g dry weight) for a period of 60 days. Cognitive performance of fish was evaluated using a plus maze associative learning paradigm. Oxidative stress, as the main driver of Se neurotoxicity, was assessed by measuring the ratio of reduced to oxidized glutathione (GSH:GSSG), lipid peroxidation (LPO) levels, and mRNA expression of several antioxidant enzymes in the zebrafish brain. Dopamine levels in the brain and the expression of genes involved in dopamine synthesis, storage, reuptake, metabolism, and receptor activation were examined. Moreover, transcription of several synaptic plasticity-related immediate-early and late response genes was determined. Overall, fish fed with the two highest concentrations of dietary Se displayed impaired associative learning. Se exposure also induced oxidative stress in the zebrafish brain, as indicated by a reduction in GSH:GSSG ratio, increased LPO levels, and up-regulation of antioxidant genes in fish treated with the two highest concentrations of Se. An increase in brain dopamine levels associated with altered expression of dopaminergic cell markers was evident in different treatment groups. Moreover, Se exposure led to the down-regulation of immediate-early and late response genes in fish that exhibiting learning impairment. Taken together, the results of this study imply that the induction of oxidative stress and dysregulation of dopaminergic neurotransmission may underlie Se-induced impairment of associative learning in zebrafish.

1. Introduction

The rising incidence and prevalence of neurological and neurodegenerative diseases have become a significant public health concern worldwide. New insights into etiology of neurological disorders suggest that less than 10% of them have a genetic origin, while a gene-environment interaction is a potential candidate for deciphering the other 90% of cases (Johnson and Atchison, 2009). Trace elements have emerged as one the major concerns of environmental neurotoxicity due to their broad range of applications in daily life (e.g., food, dietary supplements, cosmetics, glass, plastics, and paints) and their adverse health effect on animals.

In recent years, selenium (Se) has become a contaminant of considerable environmental importance due to its paradoxical role in nature. As an essential trace element, Se is an integral component of many selenoproteins, which have crucial roles in many biological functions, such as antioxidant defense, neurodevelopment, neuroprotection, synthesis of thyroid hormones, and reproduction (Chen and Berry, 2003; Mehdi et al., 2013). However, Se becomes extremely toxic at concentrations slightly above its bio-essential level (Janz et al., 2010). Although Se is naturally present in the

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environment at trace amounts (e.g., in rocks, shales, coal deposits, surface water, and vegetation), anthropogenic activities such as agriculture, coal combustion, and metal mining and refining contribute to Se contamination particularly in the aquatic ecosystems (Chapman et al., 2010; Wang and Becker, 2013). Selenium contamination of the aquatic ecosystems has frequently been reported in many parts of the world, especially in North America. Selenium bioaccumulation occurs in different components of the food chain such as aquatic plants and aquatic invertebrates in Se-impacted ecosystems and subsequently affects predatory animals such as fish (Hamilton, 2004; Muscatello and Janz, 2009). Indeed, this metalloid is among the most toxic elements to fish (Janz et al., 2010). There is now a general consensus that Se toxicity stems from its ability to promote the generation of reactive oxygen species (ROS) and thereby inducing oxidative stress, which can bring about developmental abnormalities, reproductive failure, and also death (Janz et al., 2010; Letavayová et al., 2006). Oxidative stress has long been considered to be a key driving factor of neurological diseases and cognitive decline in animals (Dias et al., 2013; Praticò et al., 2002). Selenium by virtue of its pro-oxidant properties can also lead to the development of several neurological disorders in both humans and animals (Ellwanger et al., 2016; Estevez et al., 2014; Vinceti et al., 2010, 2013). Since Se can be a double-edged sword with both toxic and beneficial properties, there is a growing interest on the outcome of exposure to various chemical forms of Se on the central nervous system (CNS) functions. Although low Se concentrations may have neuroprotective effects (mainly as inorganic Se), there is a growing body of evidence indicating its neurotoxic effects at a higher dose range (Vinceti et al., 2014). Disruption of several different neurotransmitter systems, such as cholinergic system, glutamatergic system, and the dopaminergic system has been suggested to be the main consequence of Se neurotoxicity (Ardais et al., 2009; Estevez et al., 2012; Naderi et al., 2017; Rasekh et al., 1997).

Dopamine (DA) is the principal monoamine neuromodulator in the CNS of all vertebrates and is implicated in the regulation of motor function, emotion, motivation, learning and memory. This neurotransmitter has also long been strongly linked to oxidative stress, neurodegenerative diseases, and cognitive disorders (Juárez Olguín et al., 2015; Meiser et al., 2013). The dopaminergic system is not only a favorable target for a variety of environmental neurotoxicants, but is also a fertile source of free radicals and oxidative stress (Jones and Miller, 2008; Meiser et al., 2013). Both enzymatic and non-enzymatic oxidation of DA itself can generate ROS, and thereby inducing oxidative damage in DA neurons (Dias et al., 2013; Meiser et al., 2013). Therefore, a delicate equilibrium between antioxidant defense and oxidative stress is crucial for dopaminergic cell viability and function.

Zebrafish (*Danio rerio*) are a valuable model in neurobehavioral research, and are increasingly used in studies investigating different aspects of learning and memory (Bailey et al., 2015). The major dopaminergic pathways and homologous receptors of mammals have been identified in the zebrafish brain (Ek et al., 2016). We have recently demonstrated that DA receptors are differentially involved in two different forms of learning and memory in zebrafish. While D1 receptor plays an important role in the associative learning task, D2 receptors mediate effects of DA on different aspects of latent learning in zebrafish (Naderi et al., 2016a, 2016b). Thus, it is reasonable to assume that the dysfunction of dopaminergic system may lead to different forms of cognitive deficit in zebrafish. In our recent study, we have reported that chronic exposure to Se can adversely affect the dopaminergic system and latent learning behavior in zebrafish (Naderi et al., 2017). The present study was designed to further explore the neurobehavioral effects of dietary Se and investigated how chronic environmentally relevant exposure to this element affects associative learning performance in zebrafish. To this end, we used a plus maze associative learning paradigm following chronic exposure of zebrafish to different concentrations of organic Se in the form of selenomethionine (Se-Met) via diet. It is important to note here that dietary Se-Met is the predominant bioavailable form of Se for fish in the natural environment (Janz et al., 2014). Redox homeostasis, DA levels, and the mRNA expression of dopaminergic cell markers in the zebrafish brain were evaluated. In addition, transcript levels of neuronal activity-dependent immediate early genes (IEGs) and late expression genes involved in neural plasticity, neurogenesis, and memory formation were also assessed.

2. Materials and methods

2.1. Animals

Adult wild-type (AB) zebrafish (6 months old, 3.67 ± 0.06 cm and 0.75 ± 0.11 g) were obtained from a colony present at the R.J.F. Smith Center for Aquatic Ecology of the University of Saskatchewan. For Se-Met exposure and behavioral assessment, a total of 320 fish were transferred to the laboratory and placed immediately in twenty 30-l experimental tanks (16 fish/tank). Fish were allowed to acclimate to the new conditions for at least 3 weeks before the start of the experiment. Then, treatments were randomly allocated to tanks, with four replicate tanks per treatment. All tanks were filled with de-chlorinated tap water and maintained with continuous aeration at a target temperature of 28 ± 1 °C. The illumination of experimental tanks was provided by overhead fluorescent light tubes (23 W) mounted above the tanks, on a 14:10 h light:dark photoperiod. Fish were fed daily with flake food (Nutrafin Max flakes, Germany).

2.2. Diet preparation and exposure

Diet preparation and exposure duration were based on previous research (Chernick et al., 2016; Thomas and Janz, 2011). Diets containing varying concentrations of Se (i.e., 3, 10, 30, and 60 μg/g dry weight (dw)) were prepared by adding appropriate amounts of Se-Met (Seleno-L-methionine, purity >98%, Sigma-Aldrich, USA) to the flake food as described previously (Naderi et al., 2017). These concentrations were selected to cover a broad range of environmentally relevant Se concentrations that have previously been reported in aquatic invertebrates and small prey fish species collected from Se contaminated waters (Driedger et al., 2009; May et al., 2008; Muscatello and Janz, 2009). The control diet was made by adding deionized distilled water without Se-Met to flake food. Fish were fed with either a control diet or Se-Met-spiked diet at 5% body weight/day ration for 30 days. After 30 days, fish were fed equal portions (2.5%) of control or Se-Met spiked foods and frozen brine shrimp (Sally's, San Francisco Bay Brand Inc., USA) for a further 30 days. Fish were fed with frozen brine shrimp to improve egg production because in a companion study we also investigated effects of the maternal Se-Met transfer on the learning ability of F1 generation. Triplicate samples from each Se-Met spiked diet and three replicates from brine shrimp were collected for determination of total concentrations of Se. During the exposure, the excess food at the bottom of tanks was siphoned out, 1 h after the last feeding time. A 75% water change was performed in each day daily. On 30th and 60th day of the exposure period, water samples ($n = 3$ per treatment) were collected, filtered (using 0.45 μm disposable filters), and stored at 4 °C for quantification of dissolved Se.