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Arsenic downregulates gene expression at the postsynaptic density in mouse cerebellum, including genes responsible for long-term potentiation and depression

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HIGHLIGHTS

- Arsenic exposure decreased thickness of cerebellar post synaptic density (PSD).
- The differentially expressed genes of 20 related to LTP and LTD were screened out in cerebellum of mice exposed to arsenic.
- The expression of Gria1, Gria2, Grin1, Itpr1, Grm1, CaMKII and PLCβ4 was downregulated at the PSD among the differentially expressed genes.
- These downregulated genes and their signaling pathway may be involved in the adverse effect of arsenic-induced neurotoxicity in the cerebellum.

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ABSTRACT

Arsenic (As) is a neurotoxin induces dysfunction of learning and memory. Research has indicated that cerebellum may be involved in arsenic-induced impairment of learning and memory. However, the molecular mechanisms that underlie these effects remain unclear. This study screened for the differentially expressed genes related to the long-term potentiation and long-term depression (LTP and LTD) at the cerebellar postsynaptic density (PSD) of mice following exposure to arsenic, and we provide evidence of the mechanism by which arsenic adversely affects the functions of learning and memory. Here, SPF mice were exposed to 1 ppm, 2 ppm and 4 ppm As_2O_3 for 60 days. The ultrastructure of the synapses in cerebella of these mice was observed via transmission electron microscopy. The cerebellum global gene expression of mice exposed to 4 ppm As_2O_3 was determined through GeneChip analysis. We used the web tool DAVID to analyze the Gene Ontology (GO) and KEGG pathways that were significantly enriched among the differentially expressed genes. Our observations of synaptic ultrastructure showed that the thickness of the cerebellar PSD was reduced in mice exposed to arsenic. Go analysis revealed the PSD as a significantly altered cellular component. KEGG pathway analysis showed that LTP and LTD were affected by arsenic with highest statistical significance, and 20 differentially expressed genes were associated with them. Among these differentially expressed genes, significant decreases in the mRNA expressions of CaMKII, Gria1, Gria2, Grin1, Itpr1, Grm1 and PLCβ4 related to the LTP and LTD were found at the PSD of mouse cerebellum exposed to arsenic. The downregulation of these genes was further confirmed via real-time reverse transcription PCR or Western blot at 1 ppm, 2 ppm and 4 ppm As_2O_3. Our results indicate that the 7 genes with in cerebellar PSDs may be involved in arsenic-induced neurotoxicity, including impairment of learning and memory.

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1. Introduction

Arsenic is an important neurotoxic metalloid present in our environment. Environmental exposure to arsenic has become a global health problem affecting millions of people. In arsenic-contaminated areas, the concentration in drinking water or groundwater generally ranges from 0.25 to 2.1 ppm and can be greater than 3.0 ppm in some severely contaminated areas (Chen et al., 1962; Chowdhury et al., 2000; Rahman et al., 1998). It has been reported that arsenic causes a wide array of adverse health effects, including respiratory, gastrointestinal, hematologic, hepatic, renal, dermic, immunologic and neurologic effects (Rodriguez et al., 2003; Duker et al., 2005). Epidemiological studies have revealed that chronic exposure to inorganic arsenic via drinking water results in a dose-dependent reduction in the intellectual functions of children (Wasserman et al., 2004; Wright et al., 2006). In animals exposed to arsenic, deficits in learning tasks and changes in behavior have been observed (Nagaraja and Desiraju, 1994; Zhang et al., 1999; Rodríguez et al., 2001). Taken together, these findings indicate that arsenic induces neurotoxic effects in the central nervous system, including impairing learning and memory.

It has been reported that arsenic exposure causes defects in the cerebellum (Namgung et al., 2003). In a previous study, we also showed that arsenic damages cerebellar neurons (Piao et al., 2005). Wang et al. (2009) found that the arsenic content in the cerebellum was significantly greater in arsenic-exposed mice compared with the control group, and increased in a dose-dependent manner. These findings indicated that arsenic can accumulate in the cerebellum and that the increased level of arsenic could be responsible for its neurotoxic effects. Some literatures have documented that the cerebellum contributes both to motor functions and to some sensory, cognitive, linguistic and emotional aspects of behavior (Leiner et al., 1991; Schmahmann, 1998; Timmann, 2012). The cerebellum could be involved in the arsenic-induced impairment of learning and memory. However, the molecular mechanisms by which arsenic adversely affects learning and memory remain poorly understood.

Long-term synaptic plasticity is believed to be the molecular basis of learning and memory (Bliss and Collingridge, 1993; Kandel, 2001). Long-term potentiation and long-term depression (LTP and LTD) are two forms of synaptic plasticity and have been demonstrated to occur in most brain regions in rodents as well as in slices from the human brain (Chen et al., 1996). LTP and LTD are believed to occur also in the cerebellar cortex (Zhu et al., 2007). Long-term synaptic plasticity regulates the strength of synaptic transmission. LTP increases, whereas LTD decreases synaptic transmission (Linden, 1999; Normann et al., 2000). It is known that a persistent change in the efficacy of the synaptic transmission is the basic mechanism underlying learning and memory. The postsynaptic density (PSD) is a protein-dense specialization attached to the postsynaptic membrane consisting of a massive multi-protein complex, whose functions include positioning signaling molecules for the induction of LTP and LTD related to synaptic strength. Hundreds of proteins have been identified in the PSD, including glutamate receptors, scaffold proteins, kinases and many signaling molecules (Sheng and Hoogenraad, 2007). Moreover, many proteins in the PSD are involved in the regulation of synaptic functions (Kim and Sheng, 2004). Therefore, abnormal changes in the structure and components of the PSD may disturb normal synaptic plasticity and synaptic transmission and ultimately lead to neurological disorders. It has been reported that many neurotoxicants, including lead, aluminum and fluoride, can disturb the expression of genes encoding important receptors or signaling molecules at PSDs in the brain, and the impaired expression of these genes might be involved in neurotoxicity, including the impairment of learning and memory (Nihei and Guilarte, 2001; Jing et al., 2004; Ampuero et al., 2010). This suggests that the genes encoding many proteins in the PSD region may be the targets of neurotoxins.

We hypothesize that arsenic exposure could interfere with the expression of genes at the PSD along with related signaling pathways in the cerebellum. Ultrastructure changes of cerebellar PSDs were observed in mice exposed to 1, 2 and 4 ppm As_2O_3 subchronically via transmission electron microscopy. The expression profiles of genes expressed in the cerebella of mice exposed to arsenic were determined through GeneChip analysis. Moreover, the web tool DAVID was used to analyze the significantly enriched gene ontology (GO) and KEGG pathways in the differentially expressed genes. Seven differentially expressed genes related to the LTP and LTD at PSD were screened out and identified by real-time quantitative PCR. Among the 7 differentially expressed genes, Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and phospholipase Cβ4 (PLCβ4), are important regulators related to the LTP or LTD. Therefore, we further analyzed the influence of arsenic on CaMKII and PLCβ4 within cerebellar PSDs by Western blot. We aimed at screening the differentially expressed genes related to the LTP and LTD at cerebellar PSDs of mice exposed to arsenic and providing evidences for exploring the mechanisms that arsenic affects adversely the function of learning and memory.

2. Materials and methods

2.1. Animals and treatments

Sixty-four SPF mice (age, 9 weeks) weighing 26.3–30.9 g were purchased from the Experimental Animal Center of Dalian Medical University. They were housed five per cage under standard conditions, with a 12 h dark-light cycle, at 18 °C–22 °C and 50% humidity and were maintained on a standard diet with water available ad libitum. All of the animals were randomly assigned to four groups according to their body weight. Group 1 orally received double-distilled water alone as a control. Groups 2–4 orally received double-distilled water containing As_2O_3 at a dose of 1, 2, and 4 ppm, respectively. All treatments were continued for 60 days. The experiments were performed in accordance with the Animal Guidelines of Dalian Medical University and in agreement with the Ethical Committee of Dalian Medical University.

2.2. Sample collection

Sixty days after treatment, all of the animals were weighed. For ultrastructural analyses, four mice were randomly selected from each group and anesthetized through the administration of an overdose of sodium pentobarbital, followed by transcardial perfusion with glutaraldehyde. After approximately 1 h of perfusion, the mice were decapitated, and their cerebella were removed, then rapidly cut into thin slices and fixed in glutaraldehyde at 4 °C until use. For gene and protein analyses, the remaining twelve mice in each group were euthanized, and their cerebella were quickly removed, weighed and frozen in liquid nitrogen. Samples were stored at −80 °C until use.

2.3. PSD isolation

PSDs were isolated as described previously (Villasana et al., 2006). After decapitation, the brains were quickly removed, and the cerebellum were dissected and frozen on dry ice within 2 min. The cerebellum were subsequently homogenized using homogenization buffer without detergents (0.32 M sucrose, 1 mM $NaHCO_3$, 1 mM $MgCl_2$ and 0.5 mM $CaCl_2$), and the homogenates were centrifuged at 1,200 × g for 10 min. The pellet was washed with homogenization buffer and centrifuged at 710 × g for 10 min. The supernatant from this spin was combined with the supernatant