Form 2

Dissertation Abstract

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Dissertation title	Effects of Environmental Chemicals on Brain Development and Their Sex Differences (脳発達に対する環境化学物質の影響とその性差)		

It has been global concern about environmental contaminants that adversely affect human health. Children are more vulnerable to chemical exposure than adult, and developmental exposure often leads permanent neurodevelopmental disorders. There are several chemicals in the environment of urbanized and rural areas, which have neurotoxic and neuroendocrine toxic effects. In early days, neurons were thought to have a greater role in pathophysiologic mechanisms of neurodevelopmental disorders. However, astrocytes are recognized to have the roles in some sex-biased neurodevelopmental disorders in recent years. A few studies have shown that some environmental chemicals having endocrine destructive potency affect sexually dimorphic behavior and cognitive functions in sex specific manners. The present study aimed to examine the effects of environmental toxic chemicals on primary culture astrocytes and to determine the mechanisms of toxicity and endocrine disrupting potency in each sex.

Arsenic contamination in ground water is a serious concern. Sodium arsenite ($NaAsO_2$) is a neurotoxicant affecting the viability and morphogenesis of neuron and altering the cell cycle of astrocytes by forcing unscheduled S-phase entry. However, the fate of astrocytes after S-phase entry and the mechanism involved in unscheduled-S-phase-entry-induced cell death remain unclear. The present study performed a live imaging analysis of cortical astrocytes obtained from fluorescent ubiquitination-based cell cycle indicator (Fucci) transgenic mice to monitor the cell fate after S-phase entry. The amount of apoptotic nucleosome and the mRNA expression of molecules relevant to unscheduled-S-phase-entry-coupled apoptosis (p53, Gm36566, p21, E2F1 and E2F4) were also measured to explore the mechanism. It was found that most cells exposed to 4 μM of $NaAsO_2$ died after unscheduled early S-phase entry. However, exposure to $NaAsO_2$ did not alter the amount of apoptotic nucleosome and failed to alter the mRNA expression of molecules related with unscheduled-S-phase-entry-coupled apoptosis. These findings suggest that $NaAsO_2$ affects the cell cycle and viability of astrocytes by inducing unscheduled-S-phase-entry-coupled cell death, which occurs with the mechanism other than apoptosis.

Next, the present study determined whether $NaAsO_2$ induces reactive astrogliosis by altering proliferation and the expression of cytoskeletal genes involved in reactive astrogliosis. The mRNA levels of glial fibrillary acidic protein (GFAP), vimentin, nestin and synemin of astrocytes were measured after exposure

to $NaAsO_2$ (0, 4, 8, 16 and 20 μM). Proliferation of astrocytes after $NaAsO_2$ exposure was examined by genomic PCR for the GFAP gene. $NaAsO_2$ suppressed the mRNA expression of GFAP, vimentin and nestin in concentration-dependent manner in both sexes, although the proliferation or viability of astrocytes was not affected by $NaAsO_2$. Consequently, astrocytes may not transform into reactive phenotype, which is a defensive reaction of astrocytes in response to the insults of the central nervous system. There was significant sex difference in the copy number of the GFAP gene and mRNA expression of GFAP and synemin independently of $NaAsO_2$ treatment. $NaAsO_2$ may not exhibit sex-biased effect on astrocytes, though cortical astrocytes exhibit sex differences in proliferation and the mRNA expression of GFAP and synemin.

Tris-(2,6-Dimethylphenyl) phosphate (TxP), a flame retardant, is recently recognize as a pollutant of indoor dust. TxP exposure may endanger developing children, because TxP has estrogenic endocrine disruptive potency. This study aimed to determine the effects of TxP on the sexual differentiation of sexually dimorphic nuclei, the principal nucleus of the bed nucleus of the stria terminalis (BNSTp) and calbindin-sexually dimorphic nucleus (CALB-SDN). The number of calbindin- immunoreactive cells and volume of the CALB-SDN were significantly larger in females by high and low doses of TxP, although the morphology of the CALB-SDN in male was affected only by low dose TxP. In addition, TxP exposure increased the number of CB-immunoreactive cells of the female BNSTp, although the morphology of the male BNSTp was unaltered. Taken together, TxP exposure masculinized the CALB-SDN completely and the BNSTp partially in females. Low dose of TxP may hypermasculinize the male CALB-SDN. The findings show that TxP disturbs the sexual differentiation of the brain. Chemicals expressing endocrine disruptive property may exhibit their effects differently between sexes.

In summary, the present study showed that $NaAsO_2$ exposure induces unscheduled-S-phase-entry-coupled cell death in cortical astrocytes. Astrocytes may not transform into reactive phenotype in response to $NaAsO_2$ exposure, and the effects of $NaAsO_2$ on cortical astrocytes are not different between sexes. However, astrocytes exhibit sex difference in proliferation and the mRNA levels of GFAP and synemin. TxP may affect the sexual differentiation of the brain by altering the morphology of the BNSTp in female mice and the CALB-SDN in mice of both sexes.