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Prenatal low-dose methylmercury exposure impairs neurite outgrowth and synaptic protein expression and suppresses TrkA pathway activity and eEF1A1 expression in the rat cerebellum

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ABSTRACT

Methylmercury (MeHg) is a highly neurotoxic environmental chemical that can cause developmental impairments. Human fetuses and neonates are particularly susceptible to MeHg toxicity; however, the mechanisms governing its effects in the developing brain are unclear. In the present study, we investigated the effects of prenatal and lactational MeHg exposure on the developing cerebellum in rats. We demonstrated that exposure to 5 ppm MeHg decreased postnatal expression of pre- and postsynaptic proteins, suggesting an impairment in synaptic development. MeHg exposure also reduced neurite outgrowth, as shown by a decrease in the expression of the neurite marker neurofilament H. These changes were not observed in rats exposed to 1 ppm MeHg. In order to define the underlying mechanism, we investigated the effects of MeHg exposure on the tropomyosin receptor kinase (Trk) A pathway, which plays important roles in neuronal differentiation and synapse formation. We demonstrated suppression of the TrkA pathway on gestation day 20 in rats exposed to 5 ppm MeHg. In addition, down-regulation of eukaryotic elongation factor 1A1 (eEF1A1) was observed on postnatal day 1. eEF1A1 knockdown in differentiating PC12 cells impaired neurite outgrowth and synaptic protein expression, similar to the results of MeHg exposure in the cerebellum. These results suggest that suppression of the TrkA pathway and subsequent decreases in eEF1A1 expression induced by prenatal exposure to MeHg may lead to reduced neurite outgrowth and synaptic protein expression in the developing cerebellum.

1. Introduction

Methylmercury (MeHg) is a potent environmental toxicant that causes neurological and developmental impairments in both humans and animal models. Human fetuses and neonates are particularly vulnerable to MeHg-induced brain damage and are sensitive even low levels of exposure (Grandjean et al., 1997; Knobeloch et al., 2007). MeHg may be transmitted to the fetus through the placenta and to infants through breast milk (Kajiwara et al., 1996; Sunberg et al., 1999; Sakamoto et al., 2002; Björnberg et al., 2005). Although several studies have evaluated the effects of early MeHg exposure on neurological development (Onishchenko et al., 2008; Hashimoto-Torii et al., 2014), little is known of the specific mechanisms of low-dose MeHg neurotoxicity during the prenatal period.

Our previous study showed that prenatal and lactational exposure to low-dose (5 ppm) MeHg resulted in motor coordination impairments (Fujimura et al., 2012). In this study, the mercury concentration in the cerebellum was 5–7 ppm at postnatal day (PND) 1. However, the concentration of 1 ppm MeHg-treated rats showed only 1 ppm at the same time. Based on these results, we considered that the 5 ppm mercury concentration in the cerebellum at perinatal period was necessary to induce biological effects. Interestingly, while 5 ppm MeHg-treated rats demonstrated no neuropathological changes in the cerebellum, such as neuronal cell death or neuroinflammation, they showed significant decreases in expression of the presynaptic protein synaptophysin (SPP). These results suggest that prenatal low-dose MeHg exposure may impair synaptic development or homeostasis; however, the underlying mechanisms remain to be defined.

Neurotrophins, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3), are well–known regulators of neurite outgrowth and synaptic organization through their interactions with the tropomyosin receptor kinases

Abbreviations: MeHg, methylmercury; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-3, neurotrophin-3; Trk, tropomyosin receptor kinase; eEF, eukaryotic elongation factor; p70S6K, p70 S6 kinase; mTOR, mammalian target of rapamycin; siRNA, small interfering RNA; Hg, mercury; SPP, synaptophysin; IP3R1, inositol 1,4,5-trisphosphate receptor type 1; GD, gestation day; PND, postnatal day; PSD95, postsynaptic density protein 95; SNAP25, synaptosomal-associated protein 25.

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(Trks) A, B, and C (Poo, 2001; Yoshii and Constantine-Paton, 2010; Takahashi et al., 2011). One mechanism by which neurotrophins affect neurite outgrowth is the regulation of protein synthesis. Eukaryotic elongation factors (eEFs) are essential to the elongation stage of protein synthesis; eEF1A1, eEF1A2, and eEF2 are expressed in neurons and have been shown to regulate neurite outgrowth (Khalyfa et al., 2001; Moon et al., 2004; Hashimoto and Ishima, 2011; Iketani et al., 2013). Importantly, eEF1A1 is induced by NGF in PC12 cells (Petroulakis and Wang, 2002). Protein synthesis is also regulated by p70 S6 kinase (p70S6K) and mammalian target of rapamycin (mTOR) pathway (Petroulakis and Wang, 2002; Lenz and Avruch, 2005; Bhattacharya et al., 2012).

In the present study, we exposed rats to low-dose MeHg during prenatal and lactational periods in order to investigate its effects on the expression of proteins related to neurite outgrowth and synapse assembly. Western blotting analyses revealed that prenatal exposure to low-dose MeHg decreased prenatal TrkA pathway activity and reduced the subsequent expression of eEF1A1 in developing cerebellar neurons. We further demonstrated that knockdown of eEF1A1 in differentiating PC12 cells reduced neurite outgrowth and synaptic protein expression.

2. Materials and methods

2.1. Animals study and MeHg treatment

Eighteen Wistar rats (9 females and 9 males, 10 weeks old) were obtained from Japan Clear (Tokyo, Japan) and allowed to acclimate to the housing facility for 1 week. Rats were then mated and pregnancies were confirmed. On gestation day (GD) 1, the males were removed, and the females were randomly divided into 3 groups of 3 animals each: (1) vehicle (no MeHg exposure), (2) 1 ppm MeHg, (3) 5 ppm MeHg. MeHg (Tokyo Chemical Industry Co., Tokyo, Japan) was administered in the drinking water from GD 1 through PND 21, encompassing the prenatal and lactational periods. The average daily MeHg intake was 0.05 mg/kg/day for rats in the 1 ppm group and 0.25 mg/kg/day for rats in the 5 ppm group. All

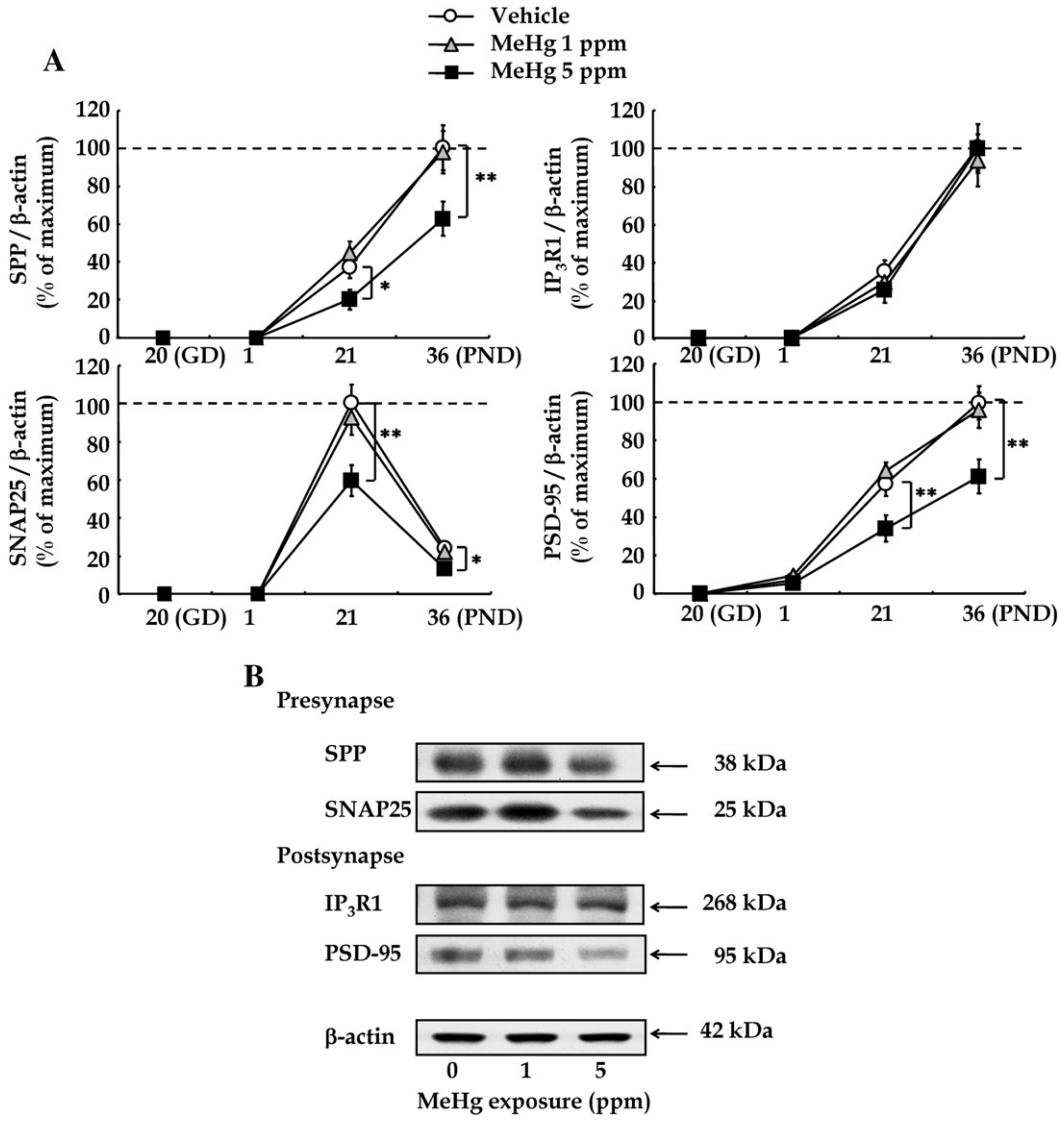

Fig. 1. Effects of MeHg exposure on synaptic protein expressions in the rat cerebellum. (a) Quantification of western blotting results for SPP, IP_3R1, SNAP25, and PSD-95 in the cerebellum of rats exposed to MeHg at 1 or 5 ppm or vehicle. Samples were collected at GD 20 through PND 36. The maximum values obtained from the cerebellum of vehicle-exposed control rats were regarded as 100% (dashed lines). Data are shown as mean ± SEM (n = 6). $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to vehicle-exposed controls. (b) Representative western blots of synaptic proteins in the cerebellum at PND 21. kDa, kilodaltons.