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The effects of methylmercury on motor activity are sex- and age-dependent, and modulated by genetic deletion of adenosine receptors and caffeine administration☆

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Abstract

Adenosine and its receptors are, as part of the brain stress response, potential targets for neuroprotective drugs. We have investigated if the adenosine receptor system affects the developmental neurotoxicity caused by the fish pollutant methylmercury (MeHg). Behavioral outcomes of low dose perinatal MeHg exposure were studied in mice where the A_1 and A_{2A} adenosine receptors were either partially blocked by caffeine treatment or eliminated by genetic modification (A_1R and A_{2A}R knock-out mice). From gestational day 7 to day 7 of lactation dams were administered doses that mimic human intake via normal diet, i.e. 1 μM MeHg and/or 0.3 g/l caffeine in the drinking water. This exposure to MeHg resulted in a doubling of brain Hg levels in wild type females and males at postnatal day 21 (PND21). Open field analysis was performed at PND21 and 2 months of age. MeHg caused time-dependent behavioral alterations preferentially in male mice. A decreased response to amphetamine in 2-month-old males pointed to disturbances in dopaminergic functions. Maternal caffeine intake induced long-lasting changes in the offspring evidenced by an increased motor activity and a modified response to psychostimulants in adult age, irrespectively of sex. Similar alterations were observed in A_1R knock-out mice, suggesting that adenosine A_1 receptors are involved in the alterations triggered by caffeine exposure during development. Perinatal caffeine treatment and, to some extent, genetic elimination of adenosine A_1 receptors, attenuated the behavioral consequences of MeHg in males. Importantly, also deletion of the A_{2A} adenosine receptor reduced the vulnerability to MeHg, consistent with the neuroprotective effects of adenosine A_{2A} receptor inactivation observed in hypoxia and Parkinson's disease. Thus, the consequences of MeHg toxicity during gestation and lactation can be reduced by adenosine A_1 and A_{2A} receptor inactivation, either via their genetic deletion or by treatment with their antagonist caffeine.

Keywords: Methylmercury; Caffeine; Adenosine receptors; Knock-out mice; Psychostimulants; Behavioral analysis

☆ In this paper the word perinatal refers to the period from gestational day 7 to postnatal day 7 in the mouse.

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1. Introduction

Methylmercury (MeHg) is a contaminant of fish and seafood with adverse effects on human cognition and behavior (Clarkson and Magos, 2006; Magos and Clarkson, 2006). As it is well recognized that the fetus and the infant are particularly susceptible to MeHg neurotoxicity (Clarkson, 1997), recommendations of limited fish intake by pregnant women have been introduced in several countries. The Joint Food and Agriculture Organization of the United Nations/World Heath Organization Expert Committee on Food Additives (JECFA) has set the provisional tolerable weekly intake to 1.6 μg/kg body weight per week (JECFA, 2003). However, more information is needed on the effects of perinatal exposure to low MeHg levels, which can be reached by frequent fish consumption during pregnancy. Epidemiological studies have shown that maternal mercury levels were inversely associated with children scores on neuropsychological tests in some populations of high fish consumers in New Zealand and Faroe Islands (Davidson et al., 2004; Grandjean et al., 1997; Kjellström et al., 1986). A recent follow-up study of a Faroe Islands cohort, characterized by a diet rich in seafood and pilot whales, have shown that children at the age of 14 years display deficits in motor, attention and verbal tests, indicating that the damage induced by methylmercury probably is permanent (Debes et al., 2006). So far no clear correlation between the effects of MeHg exposure and adverse effects has been demonstrated in young children from a fish-eating population in the Seychelles (Myers et al., 2003). However, it has been suggested that adverse effects caused by MeHg may become evident in higher cognitive functions that develop with age (Debes et al., 2006). Other factors which are present in the diet may also modulate the brain susceptibility to this neurotoxicant (Davidson et al., 2006).

In experimental animal models, gestational and lactational exposure to low doses of MeHg causes alterations of motor activity (Gímenez-Llort et al., 2001; Goulet et al., 2003; Gunderson et al., 1986, 1988; Rossi et al., 1997; Stringari et al., 2006), as well as deficits in learning and memory (Baraldi et al., 2002; Daré et al., 2003; Goulet et al., 2003). The results of several studies point to alterations in dopaminergic transmission (Bondy et al., 1979; Cagiano et al., 1990; Daré et al., 2003; Eccles and Annau, 1982; Faro et al., 2002; Gímenez-Llort et al., 2001; Lakshmana et al., 1993; Rasmussen and Newland, 2001).

The vulnerability of the brain to toxic agents depends partly on the degree of activation of natural defense mechanisms. Factors that impair the stress response to challenge by either xenobiotics or other adverse conditions, e.g. hypoxia/hypoglycemia, may aggravate toxicity. Stress conditions increase the level of the endogenous substance adenosine, which can exert a neuroprotective action via activation of four distinct G-protein-coupled (A_1, A_{2A}, A_{2B} and A_3) adenosine receptors (Fredholm et al., 2005). There are a number of reports in the literature showing that the activation of specific adenosine receptors can influence the outcome after trauma (Fredholm, 2007).

Caffeine, the most commonly used psychostimulant, acts as an antagonist of adenosine A_1 and A_{2A} receptors. It may therefore affect the vulnerability of the brain to neurotoxic substances, such as MeHg. Caffeine is widely consumed in the form of coffee, tea, cola beverages and chocolate bars even during pregnancy and lactation. Similarly to MeHg, caffeine crosses the blood–brain barrier and is excreted in milk. Newborn infants can accumulate pharmacologically active doses of caffeine because of their slow caffeine metabolism (Nehlig and Debry, 1994). However, several studies in humans have shown that consumption of moderate amounts of caffeine during pregnancy has no measurable consequences on the fetus and newborn infant (Castellanos and Rapoport, 2002; Nehlig and Debry, 1994). On the other hand, chronic maternal consumption of large quantities of coffee (more than 7 cups/day) during gestation and lactation correlated with an increased risk of intrauterine growth retardation (Fenster et al., 1991), with lower birth weight and decreased cranial circumference (Nehlig and Debry, 1994).

Increased locomotion and spontaneous activities was reported in rodents exposed to either moderate or high doses of caffeine during pregnancy/lactation (Grimm and Frieder, 1988; Holloway, 1982; Hughes and Beveridge, 1990; Nakamoto et al., 1991; Tchekalarova et al., 2005). Learning disabilities in complex visual and auditory discrimination learning paradigms have also been described, as well as delayed development of swimming (Butcher et al., 1984; Grimm and Frieder, 1988). The molecular mechanisms induced by perinatal exposure to caffeine are not known. It has been proposed that some effects may be mediated by adaptive changes in adenosine receptors/neurotransmitter systems during brain development (da Silva et al., 2005).

The present paper focuses on the role of adenosine and its receptors in modulating the developmental toxicity of the widespread environmental pollutant methylmercury. More specifically, the sensitivity to MeHg damage was studied in mice in which adenosine receptors were either partially blocked by caffeine treatment or elim-