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Cerebellum cholinergic muscarinic receptor (subtype-2 and -3) and cytoarchitecture after developmental exposure to methylmercury: An immunohistochemical study in rat

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Abstract

The developing central nervous system (CNS) is a target of the environmental toxicant methylmercury (MeHg), and the cerebellum seems the most susceptible tissue in response to this neurotoxicant.

The cholinergic system is essential for brain development, acting as a modulator of neuronal proliferation, migration and differentiation processes; its muscarinic receptors (MRs) play pivotal roles in regulating important basic physiologic functions.

By immunohistochemistry, we investigated the effects of perinatal (GD7-PD21) MeHg (0.5 mg/kg bw/day in drinking water) administration on cerebellum of mature (PD36) and immature (PD21) rats, evaluating the: (i) M2- and M3-MR expression; (ii) presence of gliosis; (iii) citoarchitecture alterations.

Regarding to M2-MRs, we showed that: at PD21, MeHg-treated animals did not display any differences compared to controls, while, at PD36 there was a significant increase of M2-immunopositive Bergmann cells in the molecular layer (ML), suggesting a MeHg-related cytotoxic effect.

Similarly to M2-MRs, at PD21 the M3-MRs were not affected by MeHg, while, at PD36 a lacking immunoreactivity of the granular layer (IGL) was observed after MeHg treatment.

In MeHg-treated rats, at both developmental points, we showed reactive gliosis, e.g. a significant increase in Bergmann glia of the ML and astrocytes of the IGL, identified by their expression of glial fibrillar acidic protein.

No MeHg-related effects on Purkinje cells were detected neither at weaning nor at puberty.

These findings suggest: (i) a delayed MeHg exposure-related effect on M2- and M3-MRs, (ii) an overt MeHg-related cytotoxic effect on cerebellar oligodendroglia, e.g. reactive gliosis, (iii) a selective vulnerability of granule cells and Purkinje neurons to MeHg, with the latter that remain unharmed.
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1. Introduction

Previous human and animal studies indicated that the developing brain in its prenatal and early postnatal stages may be at higher risk to toxic metal exposure than in adult stage (Choi et al., 1978; Harada, 1978; Amin-zaki et al., 1981; Takeuchi, 1982; Choi, 1989; Burbacher et al., 1990; Sakamoto et al., 1993, 1998, 2002, 2004; Grandjean et al., 1997). Methylmercury (MeHg), a widespread environmental neurotoxicant, originating from both natural and anthropogenic sources, bioaccumulates in organisms, easily penetrating the blood–brain and placental barriers and particularly affects brain development.

The susceptibility of central nervous system (CNS) to MeHg is well established according to epidemiological and experimental evidence (NRC, 2000; USEPA, 2001).

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Among the different brain areas, cerebellum seems to be one of the most susceptible targets in response to MeHg. Cerebellar diseases can cause intellectual impairments and aberrant behaviours; furthermore, cerebellar damage may contribute to many psychiatric disorders (Roegge and Schantz, 2006).

There are clear evidences that MeHg damages the cerebellum causing various abnormalities (Choi et al., 1978; Davis et al., 1994; Lapham et al., 1995; Eto, 1997, 2000; Roegge et al., 2006; Roegge and Schantz, 2006). Several human studies reported, within the granule cell and molecular layers, degeneration and loss of granule and Purkinje cells, increase in microglia and oligodendroglia, and damaged basket cells and parallel fibers (Matzumoto et al., 1965; Takeuchi, 1989; Roegge and Schantz, 2006; Pedersen et al., 1999).

Similarly to human data, numerous investigations in rodents have found a wide range of morphological changes in the cerebellum, following consecutive and moderate/low dose-exposure to MeHg (through gestational and lactation period), e.g. marked reduction and degeneration in Purkinje and granule cells, and focal dysplasia including heterotopic localization of Purkinje cells in the developing cerebellar cortex (Chang et al., 1977; Choi et al., 1981; Sager et al., 1984; Wakabayashi et al., 1995; Sakamoto et al., 2002). In contrast, other data demonstrated no significant MeHg (perinatal low dose) exposure-related effects on Purkinje cells neither in weanling nor in pubertal rats (Edwards et al., 2005; Roegge et al., 2006; Kakita et al., 2000).

The recent hypothesis that MeHg-related degeneration of cerebellar granule cells could be mediated by the activation of acetylcholine muscarinic receptor subtype 3 (M3) (Limke et al., 2004), further support the well-known relevance of MRs in MeHg-induced neurotoxicity.

The present study focused on the developing cholinergic system of the cerebellum after MeHg exposure. Indeed, the cholinergic system is essential for normal brain development as a modulator of neuronal proliferation and differentiation processes (Hohmann and Berger-Sweeney, 1998). The cholinergic muscarinic receptors (MRs), in particular are involved in several CNS function, including learning and memory (Levine et al., 2001). Several environmental compounds that affect the cholinergic system have been also shown to produce neurobehavioural alterations in the developing organisms (Tang et al., 2003). Recent gene-technology studies using MR-deficient mice further support the pivotal role of MRs in higher brain function (Wess, 2003).

Substantial evidences showed that cholinergic muscarinic system can be affected by in vitro and in vivo exposure to MeHg (Hrdina et al., 1976; Eldefrawi et al., 1977; Kobayashi et al., 1979; Von Burg et al., 1980; Tsuzuki, 1981; Castoldi et al., 1996, 2001; Coccini et al., 2000, 2006; Limke et al., 2004; Basu et al., 2005).

MR density has been found to be changed in the cerebellum of adult rats repeatedly administrated to low doses of MeHg (Coccini et al., 2000, 2006), as well as in mothers and their offspring exposed to MeHg low doses during pregnancy and lactation (Coccini et al., 2007). In addition, previously in vitro experiments demonstrated a direct interaction of MeHg with cerebral M1 and M2 receptors subtypes (Castoldi et al., 1996).

Cerebellar MRs are composed almost exclusively of the M2 subtype, reaching the adult values after PD35, and of the M3 subtype, expressed only in cerebellar granule cells (Limke et al., 2004; Volpicelli and Levey, 2004).

Several experiments associated MR and glial cells, being the former also implicated in the functional relationship between glial and vascular structures (Moro et al., 1995). Indeed, MRs have been detected on cerebral and cerebellar blood vessels (Grammas et al., 1983; Neustadt et al., 1988; Sato et al., 2002). Furthermore, these studies showed high degree of colocalization of MRs with glial fibrillary acidic protein (GFAP)-positive structures on astrocyte processes associated with large brain and cerebellar vessels or capillaries (Neustadt et al., 1988; Moro et al., 1995).

Typically, when the brain or cerebellum are damaged by neurotoxicants, such as MeHg, the activation of astrocytes (e.g. glyosis) can be anatomically observed (Ho et al., 2007).

The purpose of the present study was to investigate the effects of MeHg (0.5 mg/kg bw/day in drinking water) perinatally exposure from gestational day 7 to postnatal day 21 (GD7-PD21) on MR subtypes receptors and on cerebellar cytoarchitecture in weanling and pubertal rat pups.

The MeHg dose (0.5 mg/kg bw/day) was chosen based on previous observations showing (i) changes in brain MRs in adult female rats given MeHg at daily doses of 0.5–2 mg/kg/day (Coccini et al., 2000), and (ii) neurochemical and behavioural alterations in rats exposed to same dose from GD7 to PD7 (Rossi et al., 1997; Gimenéz-Llort et al., 2001).

In the present experimental model, we have taken into account the vulnerable periods of developing cerebellum, during which the basic histogenetic steps are sensitive to neuroenvironmental insults (Barone et al., 2000; Rice and Barone, 2000). In particular, it was considered the different timing of the morphogenesis of cerebellar lobulation (Altman, 1982; Doughty et al., 1998), and therefore we compared the neocerebellar lobules and paleocerebellar ones.

The specific aims of this study were to determined in weanling and pubertal rat pups (PD21 and PD36): (i) whether MeHg affects cerebellar M2 and M3 receptor subtypes, evaluated using specific antibodies; (ii) the presence of cerebellar glyosis induced by this neurotoxicant, evaluated by GFAP immunostaining; (iii) the effects on cerebellar cytoarchitecture, in particular on Purkinje cells, evaluated by histological Haematoxylin/Eosin staining and Calbindin immunostaining.

2. Materials and methods

2.1. Animals and treatment

All experimental procedures involving animals were performed in compliance with the European Council Directive 86/609/EEC on the care and use of laboratory animals.

Adult Sprague–Dawley rats (12 females and 4 males, 12 weeks old for each set of experiment) were purchased from Charles River Italia (Calco, Italy) at least 2 weeks before mating and allowed to acclimatize for 3 weeks. Throughout the experiment, animals were kept in an artificial 12 h light:12 h dark cycle with

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