Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental Research 102 (2006) 22–28
Environmental Research
www.elsevier.com/locate/envres

Cerebellar thiol status and motor deficit after lactational exposure to methylmercury

Jeferson L. Franco[a,b], Adriana Teixeira[a], Flávia C. Meotti[b], Camila M. Ribas[a], James Stringari[a], Solange C. Garcia Pomblum[c], Ângela M. Moro[c], Denise Bohrer[d], André V. Bairros[c], Alcir L. Dafre[b], Adair R.S. Santos[b], Marcelo Farina[a,*]

[a]*Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil*
[b]*Departamento de Ciências Fisiológicas, Centro de Ciências da Biológicas, Universidade Federal de Santa Catarina, 88040-900, Florianópolis, SC, Brazil*
[c]*Departamento de Análises Clínicas e Toxicológicas, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil*
[d]*Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil*

Received 31 August 2005; received in revised form 27 January 2006; accepted 8 February 2006
Available online 29 March 2006

Abstract

This study examined the exclusive contribution of methylmercury (MeHg) exposure through maternal milk on biochemical parameters related to the thiol status (glutathione (GSH) levels, glutathione peroxidase (GPx) and glutathione reductase (GR) activities) in the cerebellums of suckling mice. The same biochemical parameters were also evaluated in the cerebellums of mothers, which were submitted to a direct oral exposure to MeHg (10 mg/L in drinking water). With regard to the relationship between cerebellar function and motor activity, the presence of signs of motor impairment was also evaluated in the offspring exposed to MeHg during lactation. After the treatment (at weaning period), the pups lactationally exposed to MeHg showed increased levels of mercury in the cerebellum compared to pups in the control group and a significant impairment in the motor performance in the rotarod apparatus. In addition, these pups showed decreased levels of GSH in the cerebellum compared to pups in the control group. In dams, MeHg significantly increased the levels of cerebellar GSH and the activities of cerebellar GR. However, this was not observed in pups. This study indicates that (1) the exposure of lactating mice to MeHg causes significant impairments in motor performance in the offspring which may be related to a decrease in the cerebellar thiol status and (2) the increased GSH levels and GR activity, observed only in the cerebellums of MeHg-exposed dams, could represent compensatory pathophysiologic responses to the oxidative effects of MeHg toward endogenous GSH.

Keywords: Methylmercury; Lactational exposure; Neurotoxicity; Glutathione; Oxidative stress; Antioxidant enzymes; Motor impairment

1. Introduction

Methylmercury (MeHg) is an environmental neurotoxicant leading to neurological and developmental deficits in humans and other animals (Clarkson et al., 2003). Although MeHg-induced neurotoxicity is an extensively reported phenomenon, the molecular mechanisms underlying its toxicity are not fully understood. The major mechanisms involved in MeHg neurotoxicity currently being explored are the impairment of intracellular calcium homeostasis (Sirois and Atchison, 2000), the occurrence of oxidative stress (Ou et al., 1999), and the alteration of glutamate homeostasis (Aschner et al., 2000; Farina et al., 2003a; Manfroi et al., 2004).

During the early postnatal period, the brain is extremely sensitive to external toxins, including MeHg. At this developmental stage, a substantial acceleration of the synthesis of cerebral RNA, DNA, protein, and myelin is observed (Gottlieb et al., 1977). In particular, this period is characterized by intense gliogenesis—mainly astrocytes, a major cell type that preferentially accumulates MeHg (Aschner, 1996). In this regard, it is important to state that the exposure of pregnant women to MeHg can lead to indirect intoxication of their children (Harada, 1995; Weihe et al., 2002) and some studies indicate that fetal exposure to

*Corresponding author. Fax: +55 48 3331 9672.
E-mail address: marcelofarina@zipmail.com.br (M. Farina).

0013-9351/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2006.02.003

MeHg causes neurological deficits in offspring (Grandjean et al., 1997; Murata et al., 2004).

Even though it is well known that in utero exposure to MeHg causes neurological deficits in rats (Sakamoto et al., 2002a) and humans (Grandjean et al., 1997), evidence of the exclusive contribution of lactational exposure to MeHg as a possible cause of neurotoxicity in offspring is scarce. Indeed, studies correlating the exposure of mothers to MeHg with neurotoxic effects in offspring (Sakamoto et al., 2002a) usually do not separate these two phases of indirect exposure (transplacental and through maternal milk), making difficult to understand the real contribution of both phenomena to the development of neurotoxicity.

Oxidative stress is one of the main mechanisms related to MeHg-induced neurotoxicity. Yee and Choi (1994) showed a decrease in the activity of antioxidant enzymes and an increase in the levels of reactive oxygen species in several cellular fractions of brain of mice exposed to MeHg. In this regard, the cellular thiol status may be an important factor that affects the capability of MeHg to induce neurotoxicity. Indeed, in vitro studies have pointed to endogenous glutathione (GSH), the major intracellular sulfhydryl compound, as an important molecule responsible for protection against the neurotoxic effects of MeHg (Shanker et al., 2004). In this regard, results from our laboratory have evidenced alterations in the activity of enzymes involved in the metabolism of GSH in mice brain cortex and cerebellum after oral exposure to MeHg (Farina et al., 2005a).

Taking into account the scarcity of studies showing the real contribution of lactational MeHg exposure to the development of neurotoxicity in offspring and the relationship between MeHg-induced neurotoxicity and intracellular thiol status, this study was aimed to investigate the neurotoxic effects of exclusive lactational exposure to MeHg in suckling mice. Mercury content, GSH levels, and activities of enzymes involved in the metabolism of GSH (glutathione peroxidase (GPx) and glutathione reductase (GR)) were analyzed in the cerebellums of dams and pups. With regard to the relationship between cerebellar function and motor activity, the presence of signs of motor impairment was also evaluated in offspring exposed to MeHg during lactation.

2. Materials and methods

2.1. Chemicals

Methylmercury(II) chloride was from Aldrich Chemical Co. (Milwaukee, WI), (USA). β-Nicotinamide adenine dinucleotide phosphate sodium salt, reduced form, 5,5′-dithio-bis (2-nitrobenzoic) acid, GR from baker's yeast, and reduced GSH were obtained from Sigma (St. Louis, MO, USA). All other chemicals were of the highest grade available commercially.

2.2. Animals

Adult Swiss Albino mice (male and female), 90 days old, from our own breeding colony were maintained at 22±2 °C, on a 12:12-h light/dark cycle, with free access to food (Nuvital, PR, Brazil) and water. The breeding regimen consisted of grouping three virgin females with one male for 5 days. Pregnant mice were selected and housed individually in opaque plastic cages.

2.3. Treatment

In the first day after parturition (postnatal day 1), 14 dams were randomly assigned to one of two groups (control and Hg) of 7 animals each. Pups (eight per litter) were maintained with their mothers, which were immediately exposed to MeHg through the ingested water. Mothers from the Hg group received a solution of MeHg (10 mg/L) diluted in tap water ad libitum as sole source of liquid. MeHg dose was based on a previous study (Farina et al., 2003b). Mothers from the control group received tap water ad libitum. Thus, the exclusive route of offspring exposure to MeHg was through maternal milk. Liquid and solid ingestions of litter mothers were monitored daily. All experiments were conducted in accordance with the Guiding Principles in the Use of Animals in Toxicology, adopted by the Society of Toxicology in July 1989, and all experiments were approved by our ethics committee for animal use at the Universidade Federal de Santa Catarina (313/CEUA; 23080.026023/2004-39/UFSC).

2.4. Behavioral tests

At postnatal day 21, 2 weaning mice from each litter (7 per group) were randomly selected for the behavioral/functional tests that evaluate the animals' locomotor activity and coordination (open-field and rotarod tasks). Thus, the total number of analyzed pups was 14 per group. Initially, pups were subjected to the open-field test as previously described (Farina et al., 2005b), with minor modifications. Open-field tests were performed in a separated room with no interference noise or human activity. The locomotor activity was assessed during the treatment in sessions of 6 min using an open-field box measuring 56 (long) × 42 (wide) × 40 cm (high) with the floor divided into 12 squares. The number of squares crossed with the four paws was used as a measure of locomotor activity. After the open-field test, pups were subjected to the rotarod task which was based on the study of Duhan and Miya (1957), with minor modifications. In short, the homemade apparatus consisted of a bar with a diameter of 2.5 cm subdivided into four compartments by disks of, 25 cm with diameters. The bar rotated at a constant speed of 17 rpm and the durations (s) that the pups remained on the apparatus were recorded. Each pup was subjected to three trials and the mean of their values for remaining on the apparatus was used in the statistical analysis as actual value.

2.5. Tissue preparation

After the behavioral tests, the 2 previously selected pups (14 per group) and their respective mothers (7 per group) were killed by decapitation. Right cerebellar hemispheres were homogenized (1:5 w/v) in [*N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid)], 25 mM, pH 7.4, buffer and the tissue homogenates were rapidly centrifuged at 20,000*g* at 4 °C for 30 min. The supernatants obtained were used for the determinations of enzymatic activities, and for the quantifications of GSH contents. Left cerebellar hemispheres were used for the mercury determinations by cold vapor atomic absorption spectrometry according to Moretto et al. (2004). It is important to state that the cerebellum was the encephalic structure chosen for examination due to the high MeHg affinity by cerebellar granular cells (Klein et al., 1972).

2.6. Biochemical determinations

GR and GPx activities were determined based on Carlberg and Mannervik (1985) and Wendel (1981), respectively. GSH was measured as nonprotein thiols based on Ellman (1959) with minor modifications