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Short communication

Effects of low dose methylmercury administration during the postnatal brain growth spurt in rats

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Abstract

Male Sprague-Dawley rats from eight litters were orally administered 0.75 mg/kg/day methylmercury (MeHg) chloride from postnatal day (PD) 14 to PD 23. One male pup per litter from eight different litters per treatment group was used. Each pup was used only for a single behavioral test and tested once. The MeHg dose level resulted in Hg brain concentrations of 0.82±0.05 μg/g tissue (n=4). Locomotor behavior was studied in the Opto-Varimex apparatus by testing rats (n=8) weekly from PD 24 to PD 45. Performance of rats (n=8) on learning paradigm was analysed on PD 90. MeHg treatment induced a significant reduction in the number of rearings without altering the distance travelled, the resting time and the time spent in the central part of the arena. Results of conditioned avoidance task showed that, unlike control rats, MeHg-treated animals did not show improvement over blocks and never reached a level of performance that would indicate significant learning had taken place. The present results show that low level exposure to MeHg during late brain growth spurt induces subtle and persistent motor and learning deficits, further underlining the serious potential hazard for the exposed children.

Keywords: Methylmercury; Neurodevelopment; Locomotor behavior; Associative learning; Rat

1. Introduction

Methylmercury, an organic methylated form of mercury, is one of the most hazardous environmental pollutants. The major source of MeHg exposure to the general population is typically through consumption of contaminated fish and other food products [26]. MeHg, absorbed from the gastrointestinal tract, is easily transported across the blood-brain barrier and placenta. Once it is demethylated in the brain, elemental mercury bioaccumulates in the brain tissue [6]. Aschner et al. [1,2] have demonstrated cysteine-facilitated transport of MeHg into the brain, and they also have identified the presence of a neutral aminoacid transport system in astrocytes, which is capable of mediating MeHg-cysteine uptake. In rats, the maturation of astrocytes occurs during postnatal developmental stages [36] which correspond to the third trimester of pregnancy in humans [29].

As in humans [8,39], the developing brain in several species ranging from monkeys to mice appears far more susceptible to MeHg than the mature brain [3]. While methylmercury poisoning has been clearly associated with severe neurotoxic effects in both animal studies and human poisoning episodes, the evidence for developmental impairments associated with lower level exposure is less clear [25].

Frank human poisoning, both prenatal and postnatal, has been associated with severe mental retardation. Following exposure of the community of Minamata (Japan) and Iraqi population, the clinical course of surviving victims ranged from cases characterized by low birthweight, microcephaly, profound developmental delay, cerebral palsy, deafness, blindness and seizures [15] to ones with much milder effects including weakness, increased muscle tone, abnormal plantar reflexes, and delays in intellectual and motor development [22]. Severe neurodevelopmental effects were observed in children exposed in utero even when mothers were asymptomatic or had very mild symptoms. Despite the limitations to determine the incidence of developmental disorders

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associated with various exposure levels, the Iraqi population studies identified some dose-related effects of MeHg and raised concern that lower levels of MeHg might be associated with developmental delays [3,24].

The behavioral deficits observed in animal models of MeHg exposure also range from severe to mild depending on the exposure level. Studies reviewed by Burbacher et al. [3] show that, as in humans, also in nonhuman primates and rodents signs of severe toxicity including blindness, seizures, spasticity, quadriplegia may be observed in the most affected animals, whereas more subtle neurobehavioral deficits may be observed in the less affected ones. In this regard, MeHg effects on visual evoked potentials, swimming behavior, open field activity, motor coordination and performance of animals on various learning paradigms have been reported in rodents following pre- or postnatal exposure to this toxic compound.

Therefore, on the basis of clinical and experimental evidence, it is well established that fetuses and neonates are high-risk groups for MeHg exposure, and the MeHg effects may be more or less severe depending on the duration and level of exposure at different developmental stages [11,28]. In this regard, the implications of low level exposure are still controversial [9,21], and the lowest dose of MeHg that might impair neurodevelopment is still unknown. Another intriguing aspect is that, following prenatal or postnatal exposure to MeHg, an infant may develop psychomotor deficits as the nervous system matures. Therefore, great concern has also been raised about the long-term impact of exposure [27], since neurologic effects may continue to manifest themselves throughout life, particularly in aging population.

Concerning the critical periods of exposure during development, differences exist between humans and rodents. Rapid brain growth occurs primarily during the third trimester of pregnancy in humans, whereas in rats it occurs after parturition [38]. In particular, cerebellum as well as certain structures important in learning and memory function do not undergo extensive development until late gestation and early postnatal life [28]. While traditional views link dorsolateral prefrontal cortex with complex cognition and cerebellum with motor function, in humans and monkeys there is significant cross-talk between these two brain areas, thus implying a much closer relationship between cognitive and motor development [10].

Therefore, taking into account that the lowest dose of MeHg that might impair neurodevelopment is still unknown, the present study was primarily designed to explore, in the rat, the neurobehavioral effects of a low-level exposure to MeHg during the postnatal brain growth spurt. Taking into account that both motor and cognitive development display protracted developmental timetables and that deficits in both domains are often associated in the same developmental disorders [10], the effects of MeHg on motor and cognitive tasks were evaluated.

2. Methods

2.1. Animals and treatment schedule

The experiments have been conducted in accordance with guidelines released by Italian Ministry of Health (D.L. 116/92), the Declaration of Helsinki and the "Guide for the Care and Use of Laboratory Animals" as adopted and promulgated by the National Institutes of Health.

Primiparous Sprague-Dawley female rats (Harlan, S. Pietro al Natisone, Italy) weighing 250–280 g were used. The animals were allowed free access to food and water, were housed at constant room temperature (20–22 °C) and exposed to a light cycle of 12 h/day (08.00 h–20.00 h). Pairs of females were placed with single male rats in the late afternoon. The day on which sperm were present was designated day 0 of gestation (GD 0). Within 24 h after birth, eight litters were reduced to a standard size of eight pups per litter, and one male pup per litter from different litters was used. Pups were weaned at 24 days of age.

Male offsprings were orally administered 0.75 mg/kg/day methylmercury chloride on PD14 for 10 consecutive days. MeHg and L-cysteine (SIGMA, Milan, Italy), in a molecular ratio 1:1, were dissolved in 10% condensed milk and were administered with a microman-pipette (Gilson) for the sucklings, according to the method of Sakamoto et al. [32]. Control animals received cysteine only for 10 days. One male pup per litter from eight different litters per treatment group was used. Each pup was used only for a single behavioral test and tested once.

2.2. Mercury determination procedure

On the day after the final treatment, four MeHg-exposed rats (one rat per litter from four different litters) were deeply anesthetized by intraperitoneal injection of pentobarbital. In order to flush out blood from the brain, the rats were thoroughly perfused via the heart with physiological saline for 5 min. Then the brains were removed and kept at −80 °C until use. Total Hg concentrations were determined according to the oxygen combustion–gold amalgamation method [18], using a Mercury Analyzer MV 250R (Sugiyama-gen Environmental Science, Tokyo, Japan).

2.3. Observation of deaths, hind-limb crossing, body and brain weight monitoring

Rats were examined, weighed and deaths noted on a daily basis throughout the treatment period. The development of hind-limb crossing was also evaluated. An experimenter, blind to the animal's treatment condition, held a rat by the base of the tail for 2–5 s (perpendicular to the floor) and recorded the position of the hind limbs [20]. Results were coded with a "0" (normal) or a "1" (impaired). Brain weights were monitored on PDs 24 and 90 (one rat per litter from five different litters per tested age and per treatment group).

2.4. Locomotor activity

Motor activity was recorded in an Opto-Varimex apparatus linked to an IBM PC (Columbus Instruments, Columbus, OH) according to the method described by Wedzony et al. [35]. The apparatus consisted of a cage (42 × 42 × 30 cm) equipped with