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Prenatal Methylmercury Exposure: Effects on Stress Response During Active Learning

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Abstract The long-term impact of prenatal methylmercury (MeHg) exposure on the stress response during active learning was investigated. Pregnant rats were gavage fed MeHg (8 mg/kg) on gestational day 15. Ninety-day-old rats born to both MeHg- and saline-treated dams were subjected to an active avoidance test. The active avoidance-experienced rats (AAERs) with prenatal exposure to MeHg showed significant impairment in learning ability and exhibited higher levels of corticosterone than the untreated AAERs. The present findings suggest that the abnormal increase in plasma corticosterone levels could contribute to the poor performance of MeHg-treated AAERs in this learning task.

Keywords Methylmercury · Active avoidance task · Plasma corticosterone · Rat

One of the major sources of methylmercury (MeHg) to the general population is through the consumption of contaminated fish and other food products (NRC 2000). For fetuses, infants and children, the primary health effect of MeHg exposure is impaired neurological development (Mendola et al. 2002). There is evidence from humans and animal models that hippocampal-dependent cognition is disrupted following MeHg exposure during development (Rice and Barone 2000).

Hippocampal neurogenesis is inhibited by stress (Sapolsky 2003), possibly due to the effect of glucocorticoids (GC) on the genes involved in the cell cycle (Gould and Gross 2002). Consequently, hippocampal-dependent cognition might be impaired. This causes a great concern regarding the possible involvement of stress-induced GC secretion in MeHg-induced cognitive dysfunction. Therefore, experiments were performed to explore the effects of prenatal MeHg exposure on the stress response during active learning. For this purpose, plasma corticosterone levels were measured in undisturbed rats (UR) removed directly from their home cages and in active avoidance-experienced rats (AAER), and comparisons were made between the saline- and MeHg-treated groups. In this regard, it is well known that the active learning task, based on the avoidance of a signaled noxious stimulus, is associated with elevated plasma corticosterone levels (Scaccianoce et al. 2003).

Materials and Methods

The experiments were conducted in accordance with the guidelines of the 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 (USA).

Primiparous Sprague-Dawley female rats (Harlan, San Pietro al Natisone, Udine, Italy) weighing 250–280 g were used. The animals were allowed free access to food and

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water, housed at a constant room temperature (20–22°C) with a light cycle of 12 h/day (0800–2000) for 2 weeks before the experiment. Pairs of female rats were housed with a single male rat in the late afternoon. Vaginal smears were taken the following morning at 0900. The day on which sperms were detected was designated as gestational day (GD) 0. The pregnant rats were then randomly assigned to 2 groups: the first group was treated with saline (control [CTRL]), and the second, with 8 mg/kg MeHg on GD 15. Methylmercury chloride (Sigma-Milan, Italy) solutions were administered by intragastric intubation in volumes of 1 mL/kg of body weight. All litters were reduced to a standard size of 8 pups per litter within 24 h after birth. The pups were weaned at 21 day of age. One male pup per litter was used from the different litters of each treatment group.

Following a procedure described previously (Salmi et al. 1994), 10 adult (aged 90 days) rats born to both saline- and MeHg-treated dams were subjected to an acoustic active avoidance test. Conditioned avoidance responses (CARs) were analyzed by a two-way analysis of variance (ANOVA) for repeated measures. Tukey's or Dunnett's multiple comparison tests were used where appropriate. Because performance in the active avoidance task required intact footshock sensitivity and locomotion (Karl et al. 2003), subgroups of the CTRL and MeHg-treated rats were tested for pain threshold and motor activity.

Pain threshold was assessed using the hot plate test according to a technique previously described (Forman 2003). Reaction times (cut-off = 30 s) of the CTRL (n = 9) and MeHg-treated (n = 9) rats were analyzed by Student's *t* test.

Motor activity was recorded using an Opto-Varimex apparatus linked to an IBM PC (Columbus Instruments, Columbus, OH) according to the method described by Wedzony et al. (2000). The CTRL (n = 10) and MeHg-treated (n = 9) rats were subjected to a 20-min test (5-min session). Ambulatory time, distance travelled, and resting time were monitored using AUTOTRACK software (Columbus Instruments) and analyzed by a two-way ANOVA for repeated measures.

Immediately after the last trial of the session, 6 rats, randomly selected from the experimental groups subjected to the active avoidance test, were decapitated, and blood was collected into tubes containing 0.13 M ethylenediaminetetraacetic acid (EDTA) for measuring corticosterone concentrations. After centrifugation at 1,900*g* at 4°C for 15 min, plasma samples were separated and stored at −20°C prior to assay. Plasma corticosterone concentrations were determined using radioimmunoassay (RIA; ICN Biomedicals, Costa Mesa, CA, USA). The cross reactivity of polyclonal corticosterone-antisera with related substances was negligible. The inter- and intraassay coefficients of variation were 8% and 3%, respectively, with a detection limit of 0.0125 ng/tube. The plasma samples were diluted to 1:100 with a steroid diluent (ICN Biomedicals, Costa Mesa, CA, USA). All measurements were in the linear range of the standard curve (0.0125–3.0 ng/tube). Data were analyzed by a two-way ANOVA followed by a post-hoc test (Tukey's multiple comparison test) for comparisons between groups.

Results and Discussion

The MeHg-exposed rats exhibited impaired active learning. An overall two-way repeated measures ANOVA for CARs revealed the following differences: (i) between treatments ($F = 10.31$; $df = 1/18$; $p < 0.005$), (ii) between blocks ($F = 12.89$; $df = 3/54$; $p < 0.0001$), and (iii) between treatments × blocks ($F = 3.60$; $df = 3/54$; $p < 0.05$). Within-group comparisons (Dunnett's multiple comparison test) showed a progressive improvement in the ability of the CTRL rats to avoid the electrical shock during the training sessions (third block = $p < 0.05$; fourth block = $p < 0.01$), whereas the MeHg-treated animals failed in this task, thus demonstrating impaired learning ability. Furthermore, the Tukey's multiple comparison test showed that the MeHg-exposed offspring exhibited significant impairment in learning the active avoidance task as compared to the CTRL group (third block = $p < 0.05$; fourth block: $p < 0.01$) (Fig. 1). Since the sensitivity of the MeHg-treated rats tested using a hot plate did not differ from that of the CTRLs (*data not shown*), learning deficit

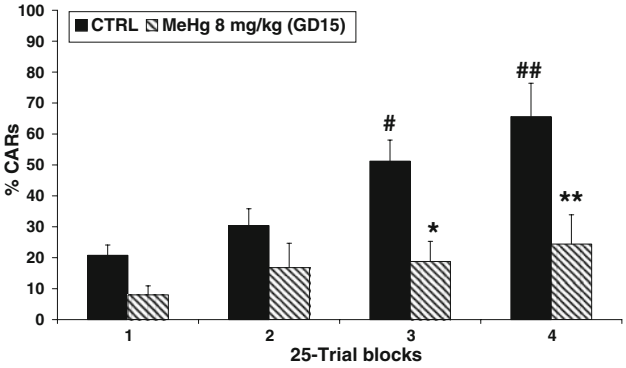

Fig. 1 Effects of prenatal MeHg exposure on active avoidance learning in 90-day-old rats. The rats were subjected to 100 trial sessions (4 blocks of 25 trials each), with a 50-s intertrial interval. The conditioning stimulus was an 80-dB tone for 10 s. The unconditioned stimulus was a 2-s positive half-wave constant current of 0.5 mA intensity. Data represent mean values ± S.E.M. $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ versus first block (Dunnett's multiple comparison test); $^{*}p < 0.05$; $^{**}p < 0.01$ versus the CTRL group (Tukey's multiple comparison test). CARs = conditioned avoidance responses; GD: gestational day; CTRL: control

cannot be attributed to sensory dysfunction. Moreover, motor disability can be also excluded. In fact, the overall two-way ANOVAs for repeated measures did not show any significant difference for ambulatory time ($F_{treatments} = 1.60$, df = 1/17, n.s.; $F_{session} = 26.06$, df = 3/51, $p < 0.0001$; $F_{treatments \times session} = 0.64$, df = 3/51, n.s.), distance travelled ($F_{treatments} = 0.57$, df = 1/17, n.s.; $F_{session} = 36.19$, df = 3/51, $p < 0.0001$; $F_{treatments \times session} = 1.03$, df = 3/51, n.s.) and resting time ($F_{treatments} = 0.006$, df = 1/17, n.s.; $F_{session} = 23.08$, df = 3/51, $p < 0.0001$; $F_{treatments \times session} = 0.36$, df = 3/51, n.s.) between the CTRL and MeHg-treated rats (*data not shown*). Finally, impairment in task acquisition could not be due to auditory loss since we have recently demonstrated that the acoustic startle response is not altered by prenatal MeHg exposure (Carratù et al. 2006). The acoustic active avoidance test implies an initial simple primary association between the cue stimuli tone and the footshock, followed by ongoing feedback experiences from subsequent trials (Stark et al. 2001). Therefore, it can be concluded that prenatal MeHg exposure affects the animals' ability to develop adaptive behavior.

The MeHg-exposed rats exhibited an abnormal increase in plasma corticosterone concentrations during active learning. An overall two-way ANOVA revealed the following differences: (i) between treatments ($F = 6.11$; df = 1/20; $p < 0.05$), (ii) between behavioral challenge ($F = 47.33$; df = 1/20; $p < 0.0001$), and (iii) between treatments × behavioral challenge ($F = 8.51$; df = 1/20; $p < 0.01$). The Tukey's multiple comparison test showed that within each treatment group, plasma corticosterone levels were significantly increased in active avoidance-experienced rats (AAERs) (CTRL: $p < 0.05$ vs. UR; MeHg 8 mg/kg: $p < 0.001$ vs. UR). Surprisingly, the AAERs exposed to MeHg exhibited a much higher increase in plasma corticosterone levels as compared to the AAERs treated with saline ($p < 0.01$) (Fig. 2). These findings highlight the impact of MeHg on the adrenocortical component of the stress response (i.e., the secretion of GCs), which could contribute to the poor performance of the MeHg-treated rats in the learning task.

The literature reveals that MeHg stimulates the hypothalamic-pituitary-adrenal (HPA) axis (Kabuto 1986; Ortega et al. 1997) and that the hepatic metabolism of corticosterone in mice exposed in utero to MeHg is reduced (Grady et al. 1978). In this context, it could be hypothesized that the learning impairment observed in the MeHg-exposed rats could be due to the treatment interference with the adrenocortical component of the stress response (i.e., the secretion of GCs) that might influence hippocampal-dependent cognition. The hippocampus is a primary GC target with a large number of corticosteroid receptors. At the most integrated level, stress or GC exposure can impair

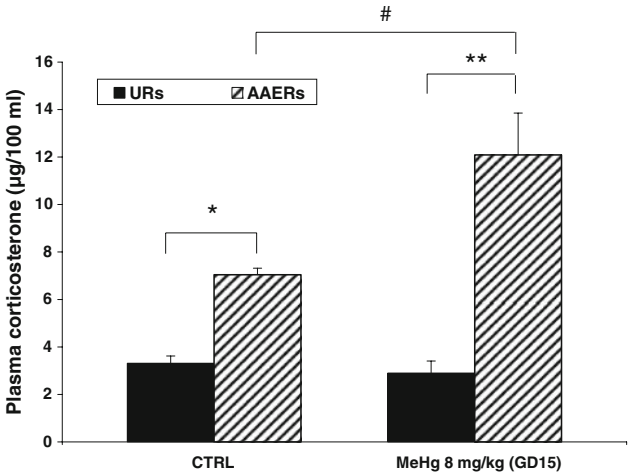

Fig. 2 Effects of prenatal MeHg exposure on plasma corticosterone concentrations in URs and AAERs. Each column represents mean values ± S.E.M. *$p < 0.05$ versus CTRL URs; **$p < 0.001$ versus MeHg-URs; $^{\#}p < 0.01$ versus CTRL AAERs (Tukey's multiple comparison test). GD: gestational day; CTRL: control; URs: undisturbed rats; AAERs: active avoidance-experienced rats

aspects of hippocampal function. The impaired performance in hippocampal-dependent tasks could be due to effects of GCs at the level of synaptic plasticity. In this regard, it is noteworthy that the occupancy of glucocorticoid receptors mediates the disruptive effects of stress and GCs upon long-term potentiation (Pavlides et al. 1993, 1995).

On the other hand, the involvement of the hippocampus in MeHg neurotoxicity is strongly supported by recent data (Falluel-Morel et al. 2007), showing that a single perinatal injection of a moderate dose of MeHg (5 µg/gbw) leads to a reduction in hippocampal size and cell number, particularly in the granule cell layer and hilus of the dentate gyrus. Correspondingly, perinatal exposure leads to profound deficits in juvenile hippocampal-dependent learning.

Although in our experimental model a different developmental window was explored, it is well known that after maternal MeHg administration, mercury bioaccumulates in the fetal brain and the peak concentration reaches the maximum within 48 hours with a decline to the normal range after weaning (Lewandowski et al. 2002). Thus, a single prenatal administration as adopted in our experimental model would result in exposure of the offspring brain spanning also the lactation period and can be expected to alter the structure and function of the hippocampus.

In conclusion, the present findings suggest that the abnormal increase in plasma corticosterone concentrations that were detected immediately after the last trial of the active avoidance session, could contribute to the poor performance of the MeHg-treated rats in the active learning task. Whether changes in neurotransmitters and/or neurotrophins are implicated in these functional deficits deserves further investigations.

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