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# Methyl Mercury Exposure During Post-Natal Brain Growth Alters Behavioral Response to SCH 23390 in Young Rats

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The brain is extremely sensitive to environmental factors during early periods of development (Annau and Cuomo 1988) and insults applied during critical stages of development may produce long lasting alterations in both biochemistry and behavior (Leviton et al. 1993; Rocha et al. 1997). In a previous study, it was demonstrated that mercury treatment during the second stage of rapid post-natal brain growth causes inhibition of the sulfhydryl- containing enzyme delta-aminolevulinic acid dehydratase in brains of suckling rats (Rocha et al. 1993; Rocha et al. 1995). Exposed rats also showed motor impairments as measured by the latency to complete a negative geotaxic response (Rocha et al. 1993). Recently, Bonnet et al, (1994) demonstrated that methymercury inhibits the specific binding of GBR 12783 to the dopamine neuronal carrier present in membranes from rat striatum, suggesting that -SH groups are involved in this inhibition. Braestrup and Andersen (1987) showed that D1 dopaminergic receptors are extremely sensitive to low concentrations of $Hg^{2+}$ and that the metal reacts with sulfhydryl groups essential for SCH 23390 binding. These data raise the possibility that the D1 dopaminergic receptors are potential molecular targets for heavy metals after in vivo exposure. Moreover, since the dopaminergic neurotransmitter system displays intense development during the early post-natal period (Broaddus and Bennet-Jr 1990) their susceptibility to environmental insults increases.

Methyl mercury is a potent neurotoxic agent produced in the environment by microbial methylation of inorganic mercury (Annau and Cuomo 1988; Atchison and Hare, 1994; Clarkson 1972; Marsh et al. 1995). Human exposure to this compound always occurs by consumption of predatory fishes (Mills et al. 1994; Weiss 1995). Once absorbed, methyl mercury may exert a variety of effects by itself or by biotransformation to inorganic mercury (Clarkson 1972; Dock et al 1995; Lind et al. 1988; Watanabe and Satoh 1996).

In the present investigation, we examined the effects of methyl mercury treatment during the second stage of rapid post-natal brain growth on the behavioral response of 25-day-old rats to SCH 23390, a specific D1 dopamine receptor antagonist. Brain levels of mercury were determined at the end of methyl mercury exposure (l&day-old rats) and after behavioral studies (26-day-old rats).

## MATERIALS AND METHODS

Wistar rats from our breeding colony were maintained on a 12-h light/dark cycle.

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The breeding regimen consisted of grouping three virgin females (90-120 days) with one male for 20 days. After this period, pregnant rats were selected and housed individually in opaque plastic cages (50 X 25 X 18 cm). Pregnant rats were checked once daily between 1500 and 1800 h for the presence of pups. When the pups were 8 days old they started to receive one daily injection (subcutaneously) of 0, 4.6, 6.9, or 9.2 mg/kg $CH_3HgCl$ dissolved in 25 mM $Na_2CO_3$ for 5 consecutive days, as previously described (Rocha et al. 1993). Some rats were killed with ether 24 h after the last injection of methyl mercury (13-day-old rats, a total of 20 rats, 5 in each group were used for mercury determination) and 24 h after behavioral measures (26-day-old rats, a total of 25 animals were used for mercury determination, 5 to 7 in each group). Brains were removed and stored at -20°C for Hg quantification. When rats were 25-day-old, they were weighed and assigned to one of the following experimental groups: 0, 0.4, or 0.8 mg/kg SCH 23390 (10 ml/kg intraperitonially).

The locomotor activity of rats were assessed in an open-field arena measuring 56 x 42 x 40 cm (high) which had the floor divided into 12 squares (Pereira et al. 1992). Each animal was subjected to 5 sessions of 2 min at 20, 50, 80, 110, and 140 min after drug administration. Locomotion was quantified by the number of squares crossed with the four paws and rearing response by the number of standing up on hind legs. Just before each open-field session, the catalepsy latency was determined as previously described by Rocha et al (1997).

The determination of mercury was made according to the method described by Emanuelli et al. (1996). Brains were removed, placed in a vial, and frozen (-20° C). Prior to mercury analysis, samples were allowed to thaw and then received 10 ml of $HNO_3$ (65%) and 1 ml of perhydrol (30%). Samples were maintained at room temperature for 24 h and heated at 45°C on a sand bath for 6 hr. Samples were filtered and volume was adjusted to 25 ml with 0.5 % $HNO_3$ (w/v). This solution was used for the determination of mercury by Atomic Absorption Spectrometry (AAS) with the aid of the cold vapor technique using a Perkin Elmer 3030 Spectrometer and a Perkin Elmer MHS-10 Hydride Generation System. All samples were run in triplicate.

Behavioral data were analyzed by a three-way ANOVA, 4 methyl mercury doses (0, 4.6, 6.9, or 9.2 mg/kg) x 3 SCH 23390 doses (0, 0.4, or 0.8 mg/kg) x 5 sessions with the session factor treated as repeated measure (repeated behavioral measurements were made on the same individuals over time). Data from brain Hg levels were analyzed by a two-way ANOVA (4 doses of methyl mercury x 2 ages). Body and brain weights were analyzed by one-way ANOVA using the mean of the litter as the experimental unit of analysis. The number of animals per group varied from 6 to 8 and an equal number of males and females were allocated to each experimental group. Statistical analysis revealed no significant effect of sex (data not shown). Each litter contributed only with 1 rat for each experimental group.

## RESULTS AND DISCUSSION

Body weight of control rats (50.4±2.1 g, n=10) was similar to those of rats exposed to 4.6 (47.9±1.5 g, n=8), 6.9 (48.7±1.4 g, n=8), and 9.2 mg/kg methyl mercury (45.8±1.9 g, n=8 ) (data expressed as mean±SEM, n = number of litters in each group). Similarly, brain weight of control rats 1,484±45 mg) was similar

from those treated with 4.6 (1,520±59 mg), 6.9 (1,456±56 mg), and 9.2 (1,437±5 mg) mg/kg methyl mercury during the second stage of rapid post-natal brain growth. Levels of mercury in brain of rats treated with methyl mercury from day 8 to 12 (Fig. 1) revealed a significant main effect of methyl mercury treatment ($F(3,37)=6.43$, $p<0.01$) due to a dose-dependent increase in mercury concentration in the brain of rats exposed to methyl mercury and also a significant methyl mercury treatment x age interaction ($F(3,37)=4.21$, $p<0.01$). The interaction was significant because the brain levels of Hg decrease from day 13 to day 26 after delivery.

Analysis of between-subject effects of methyl mercury and SCH 23390 on locomotion activity revealed a significant main effect of methyl mercury treatment ($F(3,67)=3.40$, $p<0.05$) due to reduced sensitivity of mercury-treated rats to SCH 23390 (mainly to 0.4 mg/kg, Fig. 2A). The effect of SCH 23390 administration was also significant ($F(2,67)=27.28$, $p<0.01$) due to the reduction in locomotion of rats injected with SCH 23390. Analysis of within-subject effects revealed a significant methyl mercury x SCH 23390 x sessions interaction ($F(24,268)=2.35$, $p<0.01$). This third order interaction was significant because methyl mercury-exposed rats responded less to 0.4 mg/kg SCH 23390 during the first and second open-field session (Figure 2A). The effects of methyl mercury and SCH 23390 on rearing response were similar to that obtained on locomotion activity (data not shown). The catalepsy scores of methyl mercury-treated rats injected with SCH 23390 was lower than unexposed animals which resulted in a significant methyl mercury exposure x SCH 23390 treatment interaction ($F(6,67)=7.61$, $p<0.01$). Analysis of within-subjects effect also yielded a significant methyl mercury exposure x SCH 23390 treatment x sessions interaction ($F(24,268)=6.42$, $p<0.01$) because the SCH 23390-induced increase in catalepsy scores was less pronounced in 0.4 mg/kg treated rats previously exposed to methyl-mercury from 20 to 80 min after drug administration (Fig. 2B).

Various studies have demonstrated that methyl mercury causes deregulation of dopaminergic system after in vitro or in vivo exposure (Annau and Cuomo, 1988; Bartholome et al. 1982; Bonnet et al. 1994; Kalisch and Racz 1996; Komulainen and Tuomisto 1981). The developing rat brain seems to be sensitive to methyl mercury exposure, since changes in dopamine uptake have been reported in brain of rats exposed to methyl mercury during early post-natal development (Bartholome et al. 1982). The present results indicate that methyl mercury exposure during the second stage of rapid post-natal brain growth changes the behavioral response of rats to a dopamine D1 antagonist (SCH 23390). Thus, a short period of exposure during a critical post-natal period of development seems to causes alterations in the dopaminergic system of young rats. These results are to some extent similar to those reported by others using animals exposed prenatally to methyl mercury, where an abnormal response to amphetamine was observed in adult rats (Annau and Cuomo 1988).

Methyl mercury and $Hg^{2+}$ which can be formed from methyl mercury demethylation (Lind et al, 1988; Vahter et al. 1995) have high affinity for sulfhydryl groups of proteins, including the D1 dopamine receptor and dopamine neuronal carrier protein (Bonet et al. 1994; Braestrupp and Andersen 1987).

Thus, it is reasonable to suppose that changes in the behavioral response to SCH 23390 observed in rats exposed to methyl mercury during the second stage

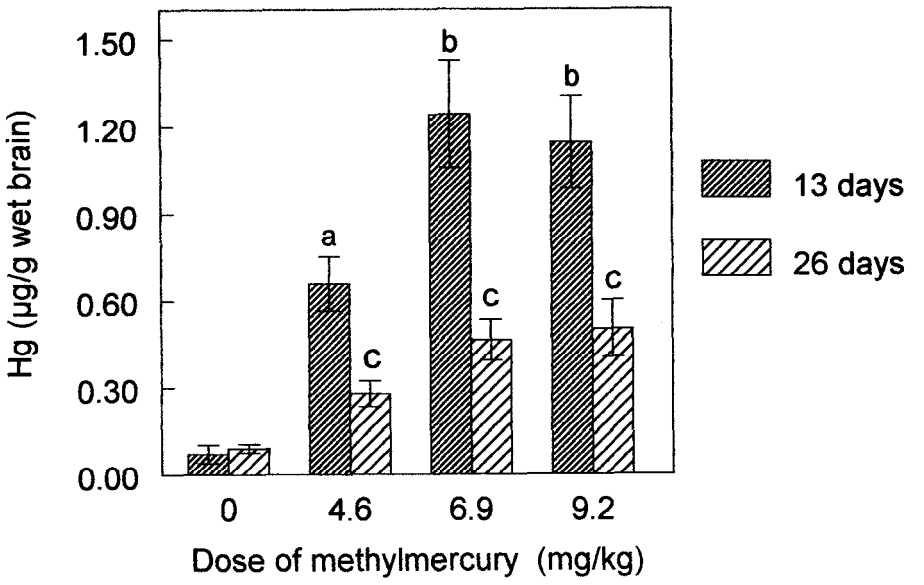


**Figure 1.** Mercury content of brain of rats at 13 and 26 days of life. The animals were intoxicated from day 8 to 12 with 0, 4.6, 6.9, or 9.2 mg/kg $CH_3HgCl$ (sc). Means not sharing the same letters are significantly different at $p<0.05$ (Duncan's multiple range test).

of rapid post-natal brain could be related, at least in part, to mercury-induced alterations in these target proteins.

In summary, the results of the present study showed that exposure to methyl mercury from day 8 to 12 of post-natal life causes a significant deposition of mercury in brain of 13-day-old rats. Although the levels of mercury in brain decreases from day 13 to day 26, the concentration is still higher than that of unexposed animals. The behavioral response to the D1 dopaminergic receptor antagonist SCH 23390 is diminished in methyl mercury exposed animals, which suggest that the dopaminergic system may be involved, at least in part, on the neurobehavioral alterations following exposure to methyl mercury during this critical stage of brain development. To our knowledge, studies dealing with MeHg exposure during the period exactly studied here are not available in the literature. Notwithstanding, O'kusky et al. (1988) exposed rats to 6.26 mg/kg MeHg from day 5 to day 15, 19 or 23; consequently, during periods that coincide with the period used in the present investigation. They reported severe changes in motor development, reduction in brain weight and on neurochemistry of exposed animals.

The relationship between the dose used in this study with the real life environment is difficult to establish. However, if we extrapolate the results of brain weights of the present study to the brain weights reported from Minamata cases disease (Takeuchi et al. 1996) we can consider the present model relevant to real life environmental contamination of methyl-mercury because it produced no gross morphological alterations, while such alterations were

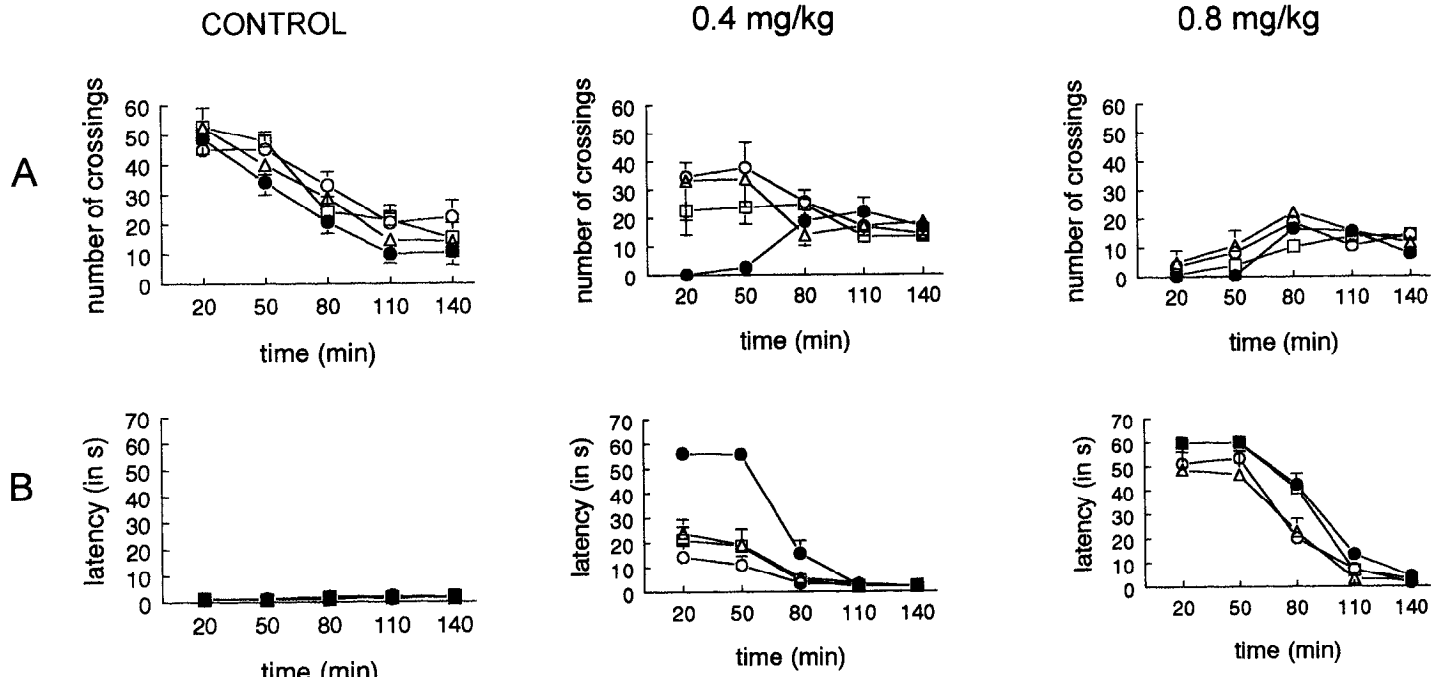


**Figure 2.** Locomotion activity (A) and catalepsy scores (B) of 25-day-old rats. Animals were intoxicated with methyl mercury as described in the Figure 1 [0 (●), 4.6 (o), 6.9 (Δ) or 9.2 mg/kg (□) $CH_3HgCl$, sc] and material and methods. At day 25 animals were injected with 0, 0.4 or 0.8 mg/kg SCH 23390 (ip) and exposed to an open-field for 2 min and catalepsy apparatus. Post-hoc comparisons were made by Duncan's multiple range test. No significant differences were found between mercury-treated groups ($p>0.10$, for all comparisons). *Indicates a significant difference from mercury-treated groups at $p<0.05$ (Duncan's multiple range test).

observed in the Minamata disease outbreak.

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## REFERENCES

Annau Z, Cuomo V (1988) Mechanism of neurotoxicity and their relationship to behavioral changes. Toxicology 49: 219-225.

Atchison WD and Hare MF (1994) Mechanism of methylmercury-induced neurotoxicity. FASEB J 8: 622-629.

Bartholome J, Trepanier P, Chait EA, Seidler FJ, Deskin R, Slotkin TA (1982) Neonatal methylmercury poisoning in the rat: effects on development of central catecholamine neurotransmitter system. Toxicol Appl Pharmacol 65: 92-99.

Bonnet JJ, Benmansour S, Amejdki-Chab N, Costentin J (1994) Effect of $CH_3HgCl$ and several transition metals on the dopamine neuronal carrier; peculiar behavior of $Zn^{2+}$. Eur J Pharmacol 266: 87-97.

Braestrup C, Andersen PH (1987) Effects of heavy metal cations and other sulfhydryl reagents on brain dopamine D1 receptors: Evidence for involvement of a thiol group in the conformation of the active site. J Neurochem 48: 1667-1672.

Broaddus WC, Bennet-Jr JP (1990) Postnatal development of striatal dopamine function. I. an examination of D1 and D2 receptors, adenylate cyclase regulation and pm-synaptic dopamine markers. Dev Brain Res 52: 265-271.

Clarkson TW (1972) The pharmacology of mercury compounds. Annu Rev Pharmacol 12: 375-406.

Dock L, Rissanen R-L, Vahter M (1995) Metabolism of mercury in hamster pups administered a single dose of $^{203}$Hg-labeled methyl mercury. Pharmacol Toxicol 76: 80-84.

Emanuelli T, Rocha JBT, Pereira ME, Porciúncula LO, Morsh VM, Martins AF, Souza DOG (1996) Effect of mercuric chloride intoxication and dimercaprol treatment on delta-aminolevulinate dehydratase from brain, liver and kidney of adult mice. Pharmacol Toxicol 79: 136-143.

Kalisch BE, Racz WJ (1996) The effects of methylmercury on endogenous dopamine eftlux from mouse striatal slices. Toxicol Lett 89: 43-49.

Komulainen H, Tuomisto J (1981) Interference of methyl mercury with monoamine uptake and release in rat brain synaptosomes. Acta Pharmacol Toxicol 48: 214-222.

Leviton A, Bellinger D, Allred EM, Rabinowitz M, Needleman H, Schoenbaum S (1993) Pre- and postnatal low-level lead exposure and children's dysfunction in school. Environ Res 60: 30-43.

Lind B, Friberg L, Nylander M (1988) Preliminary studies on methylmercury biotransformation and clearance in the brain of primates: II. Demethylation of mercury in brain. J Trace Elem Exp Med 1: 49-56.

Marsh DO, Turner MD, Smith JC, Allen P, Richdale N (1995) Fetal methylmercury study in a Peruvian fish-eating population. Neurotoxicology 16: 717-726.

Mills EL, Gutenmann WH, Lisk DJ (1994) Mercury of small pan fish from New York State waters. Chemosphere 29: 1357-1359.

Pereira ME, Huang Cl, lzquierdo I (1992) Effects of chronic treatment with 2,5-

hexanedione on several behavioral measures in rats. Psychobiology 20: 133-138.

O'Kusky JR, Boyes BE, McGeer EG (1988) Methylmercury-induced movement and postural disorders in developing rat: regional analysis of brain catecholamines and indolamines. Brain Res 439: 138-146.

Rocha JBT, Freitas AJ, Marques MB, Pereira ME, Emanuelli T, Souza DO (1993) Effects of methylmercury exposure during the second stage of rapid postnatal brain growth on negative geotaxis and on Gaminolevulinate dehydratase of suckling rats. Braz J Med Biol Res 26: 1077-1083.

Rocha JBT, Pereira ME, Emanuelli T, Christofari RS, Souza DO (1995) Effect of treatment with mercury chloride and lead acetate during the second stage of rapid postnatal brain growth on δ-aminolevulinic acid dehydratase (ALA-D) activity in brain, liver, kidney and blood of suckling rats. Toxicology 100: 27-37.

Rocha JB, Santos JET, Rocha LK, Kleinpaul ER (1997) Undernutrition during suckling changes the sensitivity to haloperidol and chlorpromazine in two behavioral measures in weaning rats. Pharmacol Toxicol 81: 114-123.

Takeuchi T, Eto K, Kinjo Y, Hidehiro T (1996) Human brain disturbance by methylmercury poisoning, focusing on the long-term effect on brain weight, Neuro Toxicol 17: 187-190.

Vahter ME, Mottet NK, Friberg LT, Lind SB, Charleston JS, Burbacher TM (1995) Demethylation of methyl mercury in different brain sites of Macaca fascilularis monkeys during long-term subclinical methyl mercury exposure. Toxicol Appl Pharmacol 134: 273-284.

Watanabe C, Satoh H (1996) Evolution of our understanding of methylmercury as a health threat. Environ Health Perspect 104(suppl): 367-379.

Weiss B (1995) Perspectives on methylmercury as a global health hazard. Neurotoxicology 16: 577-578.