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Acute Administration of Methylmercury Changes *In vivo* Dopamine Release from Rat Striatum

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Methylmercury (MeHg) is a neurotoxic agent that produces many different effects on the living organism: Paresthesia, ataxia, muscular weakness, impairment of vision, hearing and speech (Berlin 1986); depolarization of cells (Shrivastov et al. 1976, Quandt et al. 1982); alterations in protein, DNA and RNA biosyntheses, changes in phospholipid and phosphoprotein metabolism, abnormalities in mitochondrial function, and degradation of membrane integrity (Minnema et al. 1989); and changes in several mitochondrial functions, including respiration and oxidative phosphorylation (Sone et al. 1977). The mitochondria are a major site of MeHg action (Atchison 1987; Levesque and Atchison 1987).

MeHg is lipophilic and readily diffuses across cell membranes (Lakowicz and Anderson 1980). In synaptosomes, MeHg produces a disruption of synaptosomal membrane integrity and an increase in membrane permeability (transient and reversible or only a small fraction of the synaptosomes become leaky; Minnema et al. 1989). Also consistent with an increase in membrane permeability, and the membrane depolarization that ensues, is the possibility that MeHg alters the conduction of the axonal action potential (Traxinger and Atchison 1987).

The organic forms of mercury, especially MeHg, are more toxic to living organisms than the inorganic forms (Clarkson 1972). Adsorption of MeHg occurs mainly in the gastrointestinal tract (Glockling et al. 1977). Because of its high lipid solubility, MeHg penetrates the blood-brain barrier and cell membranes more readily than the inorganic forms (Felton et al. 1972).

Organic mercury affects mainly the brain, where irreversible toxic effects appear within days of exposure (Yoshino et al. 1966). However, there are no long-term studies on the effects of MeHg on neurotransmission. In vitro MeHg exposure affects the release of neurotransmitters at the neuromuscular junction, increasing spontaneous transmitter release and decreasing depolarization-evoked transmitter release (Atchison 1987). In CNS tissue preparations, MeHg increases spontaneous transmitter release (Bondy et al. 1979; Komulainen and Tuomisto 1981) and this increase was found to be concentration-dependent in synaptosomes (Minnema et al. 1989).

Studies about the distribution of mercury within the CNS of mammals have been conducted after acute administrations in tissue samples using radioactive compounds of mercury (Rodier and Kates 1988). Mercury is accumulated mainly in the following CNS regions: cortex, striatum, hippocampus, brain stem, cerebellum, and spinal cord (Moller-Madsen 1994). Exposure to mercury in different forms could be associated with alterations in motor function. Because of studies indicating that nuclei associated with motor systems contain high concentrations of mercury (Moller-Madsen 1994) nuclei (e.g. striatum) were the target of our study of mercury effects on the brain.

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The aim of our work was to study the effects of an acute administration of MeHg on the *in vivo* release of dopamine (DA) and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) from rat striatum using a microdialysis technique coupled to High-Performance Liquid Chromatography (HPLC) with electrochemical detection.

MATERIALS AND METHODS

We used female Sprague-Dawley rats (weight range: 240-260 g) housed under controled conditions of temperature (22 ± 2 °C) and photoperiod (light:dark 14:10 hr), with free access to food and water. Before the experiments, the animals were kept in polypropylene cages of 215x465x145 mm (4 rats per cage). After the administration of MeHg, each treated rat was kept in an individual cage of 220x220x145 mm.

The animals of three treated groups received via i.p. different single doses (2, 4, or 8 mg/kg) of MeHg dissolved in saline. The volume injected was 1 ml. Control animals received the same volume of saline. The number of animals was 4-5 per group.

The analysis of the acute effects of MeHg was made 48 h after its acute administration using the technique of microdialysis coupled to HPLC with electrochemical detection.

The microdialysis technique allowed the implantation of a dialysis membrane in the brain of living animals, according to a stereotaxic procedure (Westerink et al. 1990). The day after acute administration of MeHg or saline (in control rats), animals were anesthetized with chloral hydrate (400 mg/kg via i.p.) and placed in a Narishige SR-6 stereotaxic apparatus (Tokyo, Japan) for the implantation of the membranes. The skull of the animal was exposed, a guide-cannula was stereotaxically implanted in the striatum and fixed to the upper part of the skull with acrylic cement. In order to avoid the possible effects of the implantation of the guide-cannula, the operated animals were kept 24 h under controled conditions. The day after surgery (48 h after the acute administration of saline or MeHg), a CMA/12 probe with 3 mm of membrane length (CMA/Microdialysis, Sweden) was placed into the striatum inside the guide-cannula. A Ringer solution (147 mM NaCl, 4 mM KCl, 3.4 mM $CaCl_2$; pH 7.4) was forced through the probe at a flow rate of 2 µl/min by means of a CMA/102 infusion pump (CMA/Microdialysis, Sweden). The samples obtained from the microdialysis procedure were collected every 15 min (30 µl) by means of a CMA/142 microsampler (CMA/Microdialysis, Sweden). The experiments were made during 4 h with awake, conscious, and freely-moving animals, The location of the dialysis probes was verified at the end of each experiment by brain dissection.

The samples obtained from the microdialysis procedure were injected into a HP Series 1050 Liquid Chromatograph, using an injection valve Rheodyne 7125. The isocratic separation of DA and its acidic metabolites (DOPAC and HVA) was made using reversed-phase columns Spherisorb ODS-1 (10 µm particle size). The mobile phase was a mixture of acetic acid / sodium acetate, pH 4.25 with 5% methanol and 1mM EDTA, pumped at a flow rate of 1 ml/min. The detection of the substances was made by means of an ESA Coulochem model 5100A electrochemical detector (MA, USA) at a potential of +400 mV. The chromatograms obtained allowed the quantification of DA, DOPAC, and HVA with a run time of 15 min.

The results were shown as the concentration of the substances released during 15 min (30 µl sample) and they were expressed as the mean ± S.E.M. for 4-5 animals for every control or treated group. The Area Under the Curve (AUC) was calculated by the trapezoidal method. The DOPAC/DA and HVA/DA ratios were calculated using the AUC values for the control and the treated groups.

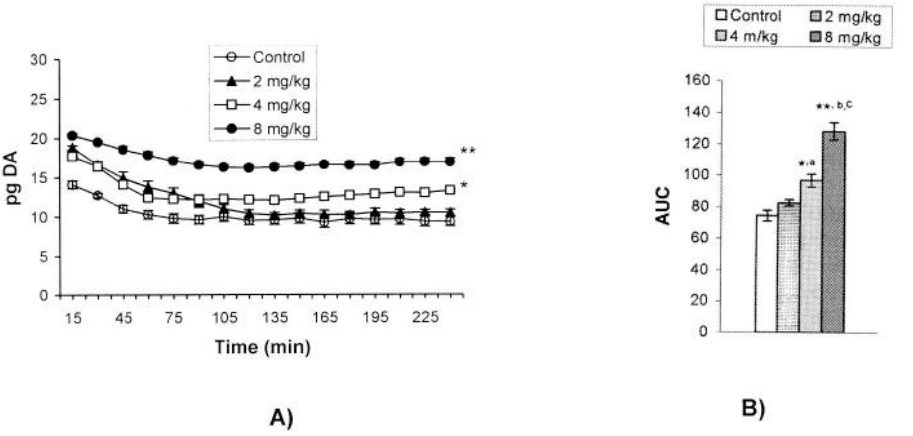


Figure. 1. **A)** Time course of the effect of the acute administration of MeHg on the release of striatal dopamine (DA) in freely moving rats. All values are the mean ± SEM (n = 4-5, in each group) of the DA release (pg of substance released during 15 min). The results are corrected according to the percentage of recovery for every microdialysis probe **B)** Effects of different doses of MeHg on the Area Under Curve (AUC) of extracellular levels of DA. Statistical analysis is performed using repeated measures ANOVA and Student-Newman-Keuls multiple range test, considering the following significant differences * $P < 0.05$, ** $P < 0.01$ as compared with control, [a]$P < 0.05$ respect to 2 mg/kg group, [b]$P < 0.01$ respect to 2 mg/kg group, [c]$P < 0.05$ respect to 4 mg/kg group.

Statistical analysis of the results was done by means of repeated measures ANOVA and Student-Newman-Keuls multiple range test, considering the significant differences between the treated groups and the control group.

RESULTS AND DISCUSSION

The results were corrected for percentage of recovery for every microdialysis probe, which was similar among probes and substances (15% for DA, 20% for DOPAC, and 25% for HVA).

The acute administration of high doses of MeHg resulted in an increase in the mortality of rats after 24 h. A dose of 16 mg/kg resulted in 75% mortality compared to 35% at 12 mg/kg. Doses of 2, 4, and 8 mg/kg did not result in any mortality of rats after 24 h.

The extracellular levels of DA in the different experimental conditions were stable 75 min after the begining of the experiment

MeHg in doses of 4 and 8 mg/kg significantly increased extracellular levels of DA in striatum compared to control (Fig. 1A). The dose of 2 mg/kg not influence DA release. The AUC values (Fig. 1B) clearly demonstrated that MeHg produced a dose-dependent increase in extracellular DA levels ([a]$P < 0.05$ comparing 4 mg/kg group with 2 mg/kg group, [b]$P < 0.01$ comparing 8 mg/kg group with 2 mg/kg group, [c]$P < 0.05$ comparing 8 mg/kg group with 4 mg/g group)

Extracellular levels of DOPAC (Fig. 2) and HVA (Fig 3) were increased by acute administration of 4 and 8 mgkg of MeHg. The acidic metabolites of DA showed similar changes to those observed with DA. It was also observed that a dose of 2 mg/kg did not produce any significant change in extracellular levels of these two metabolites. The AUC analysis showed that doses of 4 and 8 mg/kg produced significant increases in levels

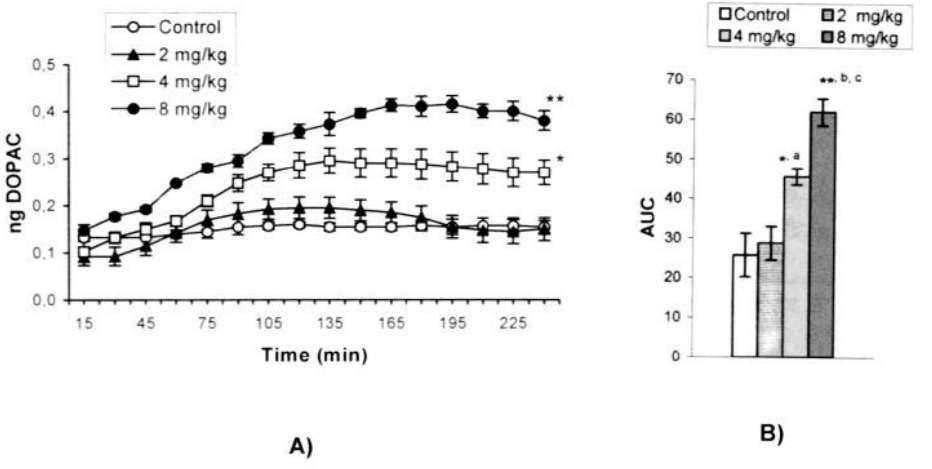


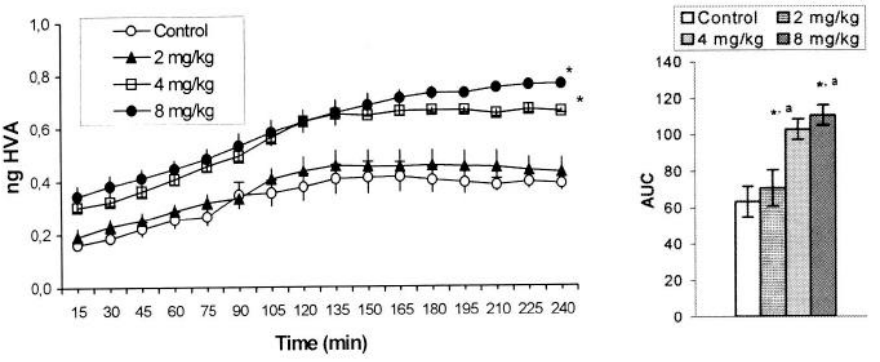
Figure. 2. **A)** Time course of the effect of the acute administration of MeHg on the release of striatal dihydroxyphenylacetic acid (DOPAC) in freely moving rats. All values are the mean ± SEM (n = 4-5, in each group) of the DOPAC release (ng of substance released during 15 min). The results are corrected according to the percentage of recovery for every microdialysis probe. **B)** Effects of different doses of MeHg on the Area Under Curve (AUC) of extracellular levels of DOPAC. Statistical analysis is performed using repeated measures ANOVA and Student-Newman-Keuls multiple range test, considering the following significant differences * $P < 0.05$, ** $P < 0.01$ as compared with control, $^{a}P < 0.05$ respect to 2 mg/kg group, $^{b}P < 0.01$ respect to 2 mg/kg group, $^{c}P < 0.05$ respect to 4 mg/kg group.

of DOPAC (77.0% and 140.8%, respectively) and HVA (62.7% and 75.4%, respectively) compared with the control.

Some possible targets for the alterations in neurotransmission induced by MeHg are the mechanisms of synthesis, release, reuptake, and degradation of neurotransmitters in nerve endings (Komulainen and Tuomisto 1981). In a previous paper (Faro et al. 1997) we discussed the effect of chronic administration of MeHg on the striatal dopaminergic system, which has been implicated in alterations of motor control such as seizures, hyperkinesia, muscular weakness and the characteristic sign named “hindlimb-crossing” (Moller-Madsen 1994).

Previous studies have shown that MeHg induced a stimulatory effect on the release of monoamines from different experimental preparations of nervous tissue (Bondy et al. 1979; Minnema et al. 1989). In some experimental conditions, MeHg also acted as an inhibitor of the reuptake of monoamines from rat brain synaptosomes (Komulainen and Tuomisto 1981). Since MeHg is lipophilic and it can readily cross the nerve terminal membrane (Miyamoto 1983), its effects on spontaneous transmitter release could be produced intraneuronally, although another possibility is the disruption of membrane integrity by MeHg (Komulainen and Tuomisto 1981). The contribution of these and other possible mechanisms of action of MeHg remains to be studied.

The increase of extracellular levels of DA that we observed could reflect stimulation of DA release or inhibition of DA reuptake. Our results seem to indicate that the uptake of DA was not inhibited because levels of DOPAC (produced from the intraneuronal degradation of DA) were increased. This suggests that the release of DA was probably increased by MeHg, however, the synthesis of DA could also have been increased by MeHg.



A) **B)**

Figure 3. **A)** Time course of the effect of the acute administration of MeHg on the release of striatal homovanillic acid (HVA) in freely moving rats. All values are the mean ± SEM (n = 4-5, in each group) of the HVA release (ng of substance released during 15 min). The results are corrected according to the percentage of recovery for every microdialysis probe. **B)** Effects of different doses of MeHg on the Area Under Curve (AUC) of extracellular levels of HVA. Statistical analysis is performed using ANOVA and Student-Newman-Keuls multiple range test, considering the following significant differences * $P < 0.05$, ** $P < 0.01$ as compared with control, $^{a}P < 0.05$ respect to 2 mg/kg group.

Several authors have reported that MeHg altered protein synthesis (Minnema et al. 1989) and the activity of some enzymes (Komulainen and Tuomisto 1981). In addition to the possible effect on monoamine release, acute administration of MeHg also could affect the activity of enzymes implicated in the degradation of DA: monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). Doses of 4 and 8 mg/kg increased extracellular levels of DOPAC and HVA, which could indicate that MeHg increased the activity of enzymes implicated in the degradation of DA without decreasing DA levels.

As DOPAC is the intraneuronal metabolite of DA (Westerink 1985) the DOPAC/DA ratio could be considered as an index of its intraneuronal degradation. We observed a significant increase in the DOPAC/DA ratio at higher doses of MeHg (table 1) indicating that MeHg induced an increase in intraneuronal metabolism of DA. However, its extraneuronal metabolism was not affected by MeHg because the HVA/DA ratio was unchanged (Table 1).

The acute effect of MeHg on the release of DA and its metabolites is dose-dependent. However, in the case of the chronic administration, we have found that the effect of MeHg on DA release seemed to be dependent on the dose and independent on the pattern of administration, whereas the effect of MeHg on extracellular levels of dopaminergic metabolites seemed to depend on the pattern of administration more than the total dose administered (Faro et al. 1997). At the present, we are using the microdialysis system for the administration of MeHg directly into the striatum (intradialysate administration) in order to define its possible direct mechanisms of action.

Table 1. Effect of the acute administration of MeHg on the DOPAC/DA and HVA/DA ratios calculated using the AUC values for the control and the treated groups. The values are the mean ± SEM. Significant differences: * $P < 0.05$ as compared with control, [a]$p < 0.05$ respect to 2 mg/kg group.

Ratio	*Control*	*2 mg/kg*	*4 mg/kg*	*8 mg/kg*
DOPAC/DA	0.35 ± 0.06	0.35 ± 0.03	0.47 ± 0.05 *, [a]	0.48 ± 0.02 *, [a]
HVA/DA	0.85 ± 0.09	0.85 ± 0.12	0.86 ± 0.04	0.87 ± 0.07

In conclusion, the acute administration of different doses of MeHg (4 and 8 mg/kg) produced significant increases on the release of DA and on the extracellular levels of its metabolites (DOPAC and HVA) from the rat striatal tissue. These increases could be due to stimulated DA release (without inhibition of its reuptake) and/or stimulation of DA intraneuronal degradation. These results are consistent with other reported studies (Faro et al. 1997).

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