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Comparative effects of organic and inorganic mercury on *in vivo* dopamine release in freely moving rats

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Abstract

The present study was carried out in order to compare the effects of administration of organic (methylmercury, MeHg) and inorganic (mercury chloride, $HgCl_2$) forms of mercury on *in vivo* dopamine (DA) release from rat striatum. Experiments were performed in conscious and freely moving female adult Sprague-Dawley (230-280 g) rats using brain microdialysis coupled to HPLC with electrochemical detection. Perfusion of different concentrations of MeHg or $HgCl_2$ (2 µL/min for 1 h, N = 5-7/group) into the striatum produced significant increases in the levels of DA. Infusion of 40 µM, 400 µM, or 4 mM MeHg increased DA levels to 907 ± 31, 2324 ± 156, and 9032 ± 70% of basal levels, respectively. The same concentrations of $HgCl_2$ increased DA levels to 1240 ± 66, 2500 ± 424, and 2658 ± 337% of basal levels, respectively. These increases were associated with significant decreases in levels of dihydroxyphenylacetic acid and homovallinic acid. Intrastriatal administration of MeHg induced a sharp concentration-dependent increase in DA levels with a peak 30 min after injection, whereas $HgCl_2$ induced a gradual, lower (for 4 mM) and delayed increase in DA levels (75 min after the beginning of perfusion). Comparing the neurochemical profile of the two mercury derivatives to induce increases in DA levels, we observed that the time-course of these increases induced by both mercurials was different and the effect produced by $HgCl_2$ was not concentration-dependent (the effect was the same for the concentrations of 400 µM and 4 mM $HgCl_2$). These results indicate that $HgCl_2$ produces increases in extracellular DA levels by a mechanism differing from that of MeHg.

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- Dopamine release *in vivo*
- Microdialysis
- Mercury determination
- Striatum

Mercury is a global pollutant considered to be a persistent bioaccumulative and toxic chemical. In its elemental inorganic form (Hg^0), mercury is transported in the atmosphere and transformed to mercuric mercury (water soluble), that is captured by the air or rain, reaching the oceans and rivers. In this form, mercury may be transformed into a stable and soluble organic form, methylmercury (MeHg), after deposition (1).

The toxic effects of mercury depend on its chemical form, dose used, duration of exposure, and route of administration. However, the organic forms, especially MeHg,

are more toxic to living organisms than the inorganic forms (2), probably, because of the high lipid solubility of MeHg. Thus, MeHg penetrates the blood-brain barrier and cell membranes more readily than the inorganic forms (3).

The mechanism by which MeHg produces neurotoxicity is poorly understood. MeHg is very reactive and has high affinity for -SH groups; this affinity is the main factor responsible for its effects but also contributes to the various mechanisms by which MeHg expresses its neurotoxicity. The MeHg-induced alterations in the nervous system may be due to the ability of the compound to disrupt synaptic transmission. It has been shown that exposure to MeHg stimulates the spontaneous release of dopamine, glutamate, acetylcholine, and amino acids *in vitro* (4-9), and dopamine *in vivo* (10).

The inorganic forms of mercury (HgCl, $HgCl_2$) have different degrees of neurotoxicity. $HgCl_2$ exerts a well-known inhibitory effect on membrane transport (11) that may cause, for example, selective inhibition of glutamate uptake by mouse astrocytes (9,12) and by cerebral cortex slices from young rats (13). Although limited studies have been reported on this form of mercury, stimulation of the spontaneous release of neurotransmitters such as dopamine and aspartate has also been observed (14).

Because MeHg and $HgCl_2$ are neurotoxic to nervous system with an influence on neurotransmission, we decided to study their effects on the dopaminergic striatal system. Thus, we compared the intrastriatal administration of $HgCl_2$ and of MeHg in order to characterize the effects of the two mercury derivatives on the *in vivo* release of dopamine (DA), the main striatal neurotransmitter, and its main metabolites, dihydroxyphenylacetic acid (DOPAC) and homovallinic acid (HVA), from the striatum of conscious and freely moving rats using a microdialysis technique coupled to HPLC with electrochemical detection.

Female adult Sprague-Dawley rats (weighing 230-280 g) were used in the experiments. Animals were housed under controlled conditions of temperature (22 ± 2ºC) and photoperiod (light:dark cycle, 14 h:10 h), with free access to food and water. All experiments were performed in accordance with the Guidelines of the European Union Council (86/609/EU) and the Spanish regulations (BOE 67/8509-12, 1988) for the use of laboratory animals.

MeHg and $HgCl_2$ (99%) were purchased from Sigma (St. Louis, MO, USA) and were dissolved in the perfusion fluid and applied locally to the striatum via a dialysis probe. All other chemicals were of analytical grade.

For microdialysis sampling, animals were anesthetized *ip* with chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus (Narishige SR-6, Tokyo, Japan) for the implantation of a guide cannula. A microdialysis probe (CMA/12,CMA Microdialysis Instruments, Solna, Sweden), 3-mm membrane length, was implanted through the guide cannula into the left striatum at the following coordinates from bregma: A/P +2.0, L +3.0, V +6.0 mm. After the experiments, rats were sacrificed with an overdose of chloral hydrate and their brains were fixed with 10% formalin via intracardiac perfusion. Coronal sections (30 µm) were stained with cresyl violet and examined to determine the exact location of the dialysis probe.

Continuous perfusion with Ringer solution (147 mM NaCl, 4 mM KCl, 3.4 mM $CaCl_2$, pH 7.4) was performed using a CMA/102 infusion pump (CMA/Microdialysis) at a flow rate of 2 µL/min. The experiments were carried out over a period of 4 h on awake, conscious, and freely moving animals, with sampling of the striatal dialysates every 15 min (30 µL).

The experiments were carried out 24 h after surgery using different concentrations of MeHg or $HgCl_2$ (40 µM, 400 µM, and 4 mM) dissolved in Ringer solution and ap-

plied locally to the striatum via a dialysis probe. After 4 basal perfusates (60 min) carried out to obtain a stable output of DA and metabolites, the striatum was perfused with the different $HgCl_2$ concentrations for 60 min. The perfusate was then switched back to the unmodified perfusion medium and the measurements were continued for an additional period of 120 min.

The samples obtained with the microdialysis procedure (30 µL) were collected with a CMA/142 microsampler (CMA/Microdialysis) and DA, DOPAC and HVA levels were quantified by HPLC with electrochemical detection. The samples obtained by the microdialysis procedure were injected into a Hewlett-Packard Series 1050 liquid chromatograph (Boston, MA, USA), using a Rheodyne 7125 injection valve (Cotati, CA, USA). The isocratic separation of DA, DOPAC, and HVA was carried out using Spherisorb ODS-1 reverse-phase columns (10-µm particle size; Deeside, UK) by the method of Durán et al. (15). The eluent, pH 4.0, was prepared as follows: 70 mM KH_2PO_4, 1 mM octanesulfonic acid, 1 mM EDTA, and 5% methanol. The flow rate was 1 mL/min. The substances were detected with an ESA Coulochem 5100A electrochemical detector (Boston, MA, USA) at a potential of +400 mV. DA, DOPAC, and HVA were separated in a run time of 15 min.

The data were corrected for recovery for every microdialysis probe, which was similar for the different probes and substances analyzed (17% for DA, 24% for DOPAC, and 24% for HVA). The mean substance concentrations in the three samples before $HgCl_2$ administration were considered as basal levels. These basal levels were considered to be 100% in order to compare the different response of DA and metabolites after $HgCl_2$ administration. Data are reported as the mean ± SEM of 4-5 experiments and expressed as percentage of basal levels.

Statistical analysis of the results was performed by repeated measures ANOVA and the Student-Newman-Keuls multiple range test, with $P \leq 0.005$ set as the level of significance.

The basal output of dialysate DA and its metabolites from the striatum (mean ± SEM) was considered as the mean of substance concentrations in the three samples before mercury perfusion as follows: DA = 0.15 ± 0.01 (N = 30), DOPAC = 16.60 ± 0.49 (N = 30), and HVA = 10.95 ± 1.13 ng/15 min (N = 30).

Different doses of MeHg produced a concentration-related increase in the striatal output of DA (Figure 1A), with maximum in-

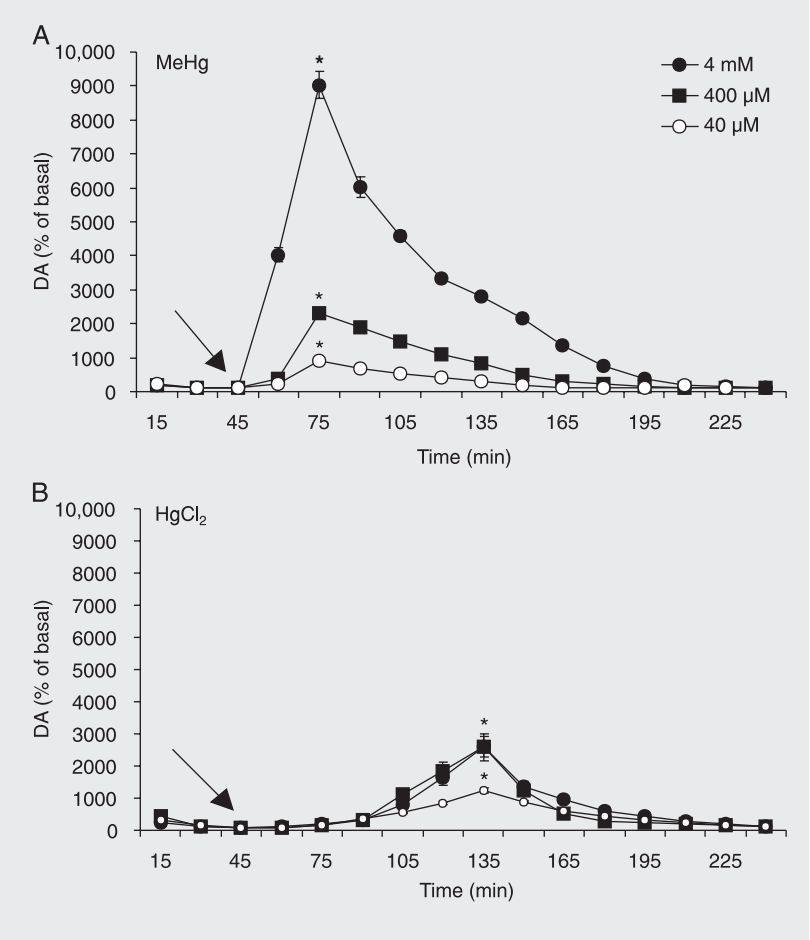

Figure. 1. Effects of intrastriatal perfusion of different concentrations (4 mM, 400 µM, 40 µM) of MeHg (A) or $HgCl_2$ (B) on extracellular dopamine (DA) levels in rat striatum. The arrow denotes the infusion of mercury for 60 min. Data are reported as mean ± SEM of 4-5 experiments per group, expressed as a percentage of basal levels (100%). Basal levels (0.15 ± 0.01 ng/15 min) were the mean substance concentrations in the three samples before $HgCl_2$ perfusion. *P < 0.05 compared to basal levels (Student-Newman-Keuls multiple range test).

creases 30 min after the beginning of MeHg perfusion. Maximum values of 907 ± 31, 2324 ± 156, and 9032 ± 70% of basal levels, respectively, were achieved. DA returned to basal values 105 min (40-µM dose), 135 min (40-µM dose), and 165 min (40-µM dose) after MeHg administration.

The highest dose of MeHg assessed (4 mM) caused a significant decrease in extracellular levels of DOPAC and HVA in the striatum 45 min after the beginning of MeHg application (a 65 ± 2.3 and 53 ± 2.2% decrease compared to basal levels, respectively; Table 1). The remaining concentrations of MeHg (40 and 400 µM) had no significant effect on the acidic metabolites of DA.

Like MeHg, the different concentrations of $HgCl_2$ used produced increases in the striatal output of DA (Figure 1B) but the maximum DA release was less than observed with MeHg. The data (Figure 1B) show that 40 µM, 400 µM, and 4 mM $HgCl_2$ produced a maximum increase 75 min after the beginning of $HgCl_2$ perfusion (1240 ± 66, 2500 ± 424, and 2658 ± 337% of basal levels, respectively). DA concentration returned to basal values 120 min after $HgCl_2$ administration.

Table 1. Effects of MeHg or $HgCl_2$ on extracellular DOPAC and HVA levels from rat striatum.

	MeHg		$HgCl_2$	
	Basal (45 min)	MeHg (90 min)	Basal (45 min)	$HgCl_2$ (135 min)
DOPAC				
40 µM	100 ± 1.2	102 ± 1.7	102 ± 0.1	109 ± 2.3
400 µM	100 ± 1.0	101 ± 1.9	101 ± 0.3	74 ± 4.3*
4 mM	100 ± 0.9	65 ± 2.3*	102 ± 1.3	3.5 ± 0.2*
HVA				
40 µM	109 ± 2.1	110 ± 0.9	99 ± 1.8	109 ± 2.0
400 µM	106 ± 2.0	90 ± 1.1	100 ± 1.5	71 ± 4.3*
4 mM	107 ± 1.5	53 ± 2.2*	98 ± 1.3	49 ± 2.3*

Data are reported as means ± SEM for 4-5 experiments, expressed as a percentage of basal levels (100%). The basal output of dialysate was considered as the mean substance concentrations in the three samples before mercury perfusion as follows: dihydroxyphenylacetic acid (DOPAC) = 16.60 ± 0.49 ng/15 min (N = 30) and homovanillic acid (HVA) = 10.95 ± 1.13 ng/15 min (N = 30).
*P < 0.05 compared to basal levels (Student-Newman-Keuls multiple range test).

The highest concentrations of $HgCl_2$ test (400 µM and 4 mM) caused a significant decrease in extracellular levels of DOPAC and HVA in the striatum 90 and 105 min after the beginning of $HgCl_2$ administration (Table 1). The lowest concentration of $HgCl_2$ (40 µM) had no significant effect on the acidic metabolites of DA.

This comparative study demonstrated that intrastriatal administration of different concentrations of MeHg produced significant increases in the release of DA from rat striatal tissue, and they were associated with significant decreases in the extracellular levels of DOPAC and HVA. The administration of all concentrations of $HgCl_2$ assessed also induced significant increases in striatal DA levels, with decreases in the extracellular levels of DOPAC and HVA.

The concentrations of mercury used in the present study were similar to those used in other *in vitro* studies (14,16). Both MeHg and $HgCl_2$ were administered *in situ* through a dialysis probe at a flow of 2 µL/min using a Ringer medium. Under these conditions, about 17% of mercury crossed the membrane and only submicromolar concentrations of mercury were achieved at the striatal site of action.

To exclude the possibility of excitotoxicity promoted by mercury administration, at the end of the intrastriatal administration of mercury we perfused a Ringer solution with a high concentration of KCl (75 mM) for 30 min. In this hyperkalemic situation the DA levels increased to 1562 ± 296% compared to basal values (data not shown). This effect was not significantly different from that observed with mercury administration and allowed us to confirm the functional integrity of the DA synapses.

Our results indicate that MeHg-induced *in vivo* DA release seems to be concentration-dependent. In contrast, regarding the effects observed after intrastriatal MeHg administration on extracellular DA release, the effect of $HgCl_2$ administration was not

dose-dependent, with the increases in DA levels induced by $HgCl_2$ being the same for 400 µM and 4 mM $HgCl_2$ (~2500%).

Another difference between the effects of the two forms of mercury was the time needed to produce the maximal increase in extracellular DA levels, which was 30 min after the beginning of administration for MeHg and 75 min for $HgCl_2$. Comparing the results obtained with MeHg and $HgCl_2$, we observed that the increases in extracellular DA levels induced by $HgCl_2$ were lower (for the 4-mM concentration) and were delayed by 45 min when compared with the increases in DA release induced by the organic form of mercury.

At least three main mechanisms can be proposed to explain the increase in extracellular DA levels induced by mercury: 1) this chemical acts by releasing DA from its storage vesicles, 2) it inhibits monoamine oxidase, 3) it inhibits DA reuptake, which is the main mechanism of elimination of the neurotransmitter in the synaptic cleft. In previous papers, we have reported that MeHg increases the *in vivo* DA release from rat striatum and that this increase occurs through an action on the DA membrane transporter inhibiting the reuptake of this neurotransmitter (10,17).

The intrastriatal administration of different concentrations of MeHg or $HgCl_2$ produced significant increases in the release of DA from rat striatal tissue, which were associated with significant decreases in the extracellular levels of DOPAC and HVA (in response to the highest concentrations). We suggest that these results are different mechanisms of action of the organic and ionic forms of mercury on DA release.

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