**In Vivo Effects of Inorganic Mercury (HgCl₂) on Striatal Dopaminergic System**

L. R. F. Faro,* J. L. M. do Nascimento,* M. Alfonso,† and R. Durán†

*Departamento de Fisiología, Centro de Ciencias Biológicas, UFPA, Belém, PA, Brazil; and †Laboratorio de Fisiología, Departamento de Biología Funcional y Ciencias de la Salud, Facultad de Ciencias, Universidad de Vigo, Vigo, Spain

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In the present study, the effects of intrastriatal administration of different concentrations (40 μM, 400 μM, and 4 mM) of inorganic mercury (HgCl₂) on the dopaminergic system of rat striatum were evaluated, using a microdialysis technique coupled to liquid chromatography-electrochemical detection. In previous studies, we discussed the effects of organic mercury (MeHg) administration on the striatal dopaminergic system on the basis of changes in the release and metabolism of striatal dopamine (DA). In the present study, it is demonstrated that intrastriatal administration of all concentrations of HgCl₂ produced significant increases in the output of DA (1240, 2500, and 2658% for the concentrations of 40 μM, 400 μM, and 4 mM HgCl₂, respectively) from rat striatal tissue, associated with significant decreases in striatal levels of its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) with the concentrations of 40 μM and 4 mM HgCl₂ (74.4 and 3.4% for DOPAC and 71.0 and 50.6% for HVA, respectively), whereas no changes in metabolite levels were observed with the concentration of 40 μM HgCl₂. These effects are explained as a result of stimulated DA release and/or changed DA metabolism. The effects of intrastriatal administration of HgCl₂ were compared with those of MeHg on DA extracellular levels.

The inorganic forms of mercury (HgCl, HgCl₂) are neurotoxic to different degrees. The main target organ of mercuric chloride (HgCl₂) is the kidney, where it is concentrated, producing renal damage and leading to death from uremia (Barnes et al., 1980). While the kidney is generally considered the critical target organ, neurological disturbances have also been found after HgCl₂ treatment, which in some cases resemble the effects of MeHg (Sirois and Atchison, 1996).

HgCl₂ exerts a well-known inhibitory effect on membrane transport that is generally attributed to its high-affinity interaction with protein sulfhydryl groups (Bondy et al., 1979). For example, submicromolar concentrations of HgCl₂ have been found to inhibit selectively glutamate uptake in mouse astrocytes (Brookes, 1988; Brookes and Kristt, 1989).

Freitas et al. (1996) reported that both organic mercury and inorganic mercury inhibit Ca²⁺-ATPase and Ca²⁺ uptake by brain microsomes with the inhibitory potency of inorganic mercury being higher than that of organic mercury, probably reflecting differences in the affinity for the sulfhydryl groups of the mercury compounds.

Furthermore, the similarity in size and charge of various mercurials, particularly HgCl₂, to those of physiological ions such as Na⁺ and Ca²⁺ suggests that they may enter the cell via ion channels (Miyamoto, 1983).

Although mercuric salts penetrate the brain poorly (Brookes, 1992), the direct intrastriatal administration of HgCl₂ avoids the impairment of access into brain striatal tissue.

Because HgCl₂ seems to be a neurotoxin with a possible influence on neurotransmission, it was decided to study its effects on the in vivo release of dopamine (DA), the main striatal neurotransmitter, and its main 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. Moreover, the effects of intrastriatal administration of HgCl₂ were...
compared with those of MeHg on striatal DA in order to determine their possible mechanisms of action.

MATERIALS AND METHODS

Female adult Sprague–Dawley rats (weighing 240–260 g) were used in the experiments. Animals were housed under controlled conditions of temperature (22 ± 2°C) and photoperiod (light: dark 14:10 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.

The HgCl₂ (99%) was purchased from Sigma (St. Louis, MO) and was dissolved in the perfusion fluid and applied locally in the striatum via the dialysis probe. All other chemicals were analytical grade.

For microdialysis sampling, animals were anesthetized ip with chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus (Narishige SR-6) for the implantation of a guide cannula. A microdialysis probe (CMA/12, 3-mm membrane length) was implanted through the guide cannula into the left striatum at the following coordinates from Bregma: A/P +2.0 mm; L +3.0 mm; V +6.0 mm. After the experiments, rats were given an overdose of chloral hydrate, and brains were made, stained with cresyl violet, and examined to determine the exact location of the dialysis probe.

Continuous perfusion was performed with a Ringer’s solution (147 mM NaCl, 4 mM KCl, 3.4 mM CaCl₂; pH 7.4) using a CMA/102 infusion pump (CMA/Microdialysis) at a flow rate set at 2 µl/min. The experiments were performed over 4 h with awake, conscious, and freely moving animals, with sampling of the striatal dialysates every 15 min (30 µl).

The initial experiments were carried out 24 h after surgery, using different concentrations of HgCl₂ (40 µM, 400 µM, and 4 mM) dissolved in Ringer’s solution and applied locally in the striatum via the dialysis probe. After four basal perfusates (60 min), for obtaining a stable output for DA and metabolites, the striatum was perfused with the different concentrations of HgCl₂ used for 60 min. Then, the perfusate was switched back to the unmodified perfusion medium and the measurements continued for an additional period of 120 min.

In another set of experiments, the effects of HgCl₂ administration on DA, DOPAC, and HVA extracellular levels were verified using a second infusion of 400 µM HgCl₂ and a third infusion of 4 mM HgCl₂ to the same animals that previously received 40 µM HgCl₂, 1 and 2 weeks after first infusion, respectively. In this case, the dialysis probe was lowered via a chronically implanted guide cannula prior to each experiment using the same experimental conditions as those described above.

The samples obtained from the microdialysis procedure (30 µl) were collected by means of a CMA/142 microsampler (CMA/Microdialysis), and DA, DOPAC, and HVA levels were quantitated by HPLC with electrochemical detection. The samples obtained from the microdialysis procedure were injected into a Hewlett-Packard Series 1050 Liquid Chromatograph, using a Rheodyne 7125 injection valve. The isotropic separation of DA, DOPAC, and HVA was carried out using Spherisorb ODS-1 reversed-phase columns (10-µm particle size) according to Durán et al. (1998). The eluent (pH 4.0) was prepared as follows: 70 mM KH₂PO₄, 1 mM octanesulfonic acid, 1 mM EDTA, and 5% methanol. The flow rate was 1 ml/min. The detection of the substances was achieved by means of an ESA Coulochem 5100A electrochemical detector (Massachusetts) at a potential of + 400 mV. The chromatograms obtained allowed the determination of DA, DOPAC, and HVA with a run time of 15 min.

The data were corrected for percentage of 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 averages of substance concentrations in the three samples before HgCl₂ administration were considered as basal levels. These basal levels were considered as 100% in order to compare the different responses of DA and metabolites after HgCl₂ administration. The results are exhibited as the means ± SEM of four to five experiments, expressed as percentage of basal levels.

Statistical analysis of the results was performed by means of repeated-measures ANOVA and the Student–Newman–Keuls multiple-range test, considering differences to be statistically significant at the following levels: *P < 0.05, **P < 0.01, and ***P < 0.001.

RESULTS

Basal Output of DA, DOPAC, and HVA

Figure 1 presents the basal levels of DA, DOPAC, and HVA the day after surgery (considered as control) and 1 week and 2 weeks later. The basal output of dialysate DA and its metabolites from the striatum (means ± SEM) before any infusion of HgCl₂ (on day 1 after cannula implantation) was as follows: DA = 0.15 ± 0.01 (ng/15 min), DOPAC = 16.60 ± 0.49 (ng/15 min), and HVA = 10.95 ± 1.13 (ng/15 min). Basal values of DOPAC did not change 1 week later, whereas HVA basal levels increased in the same period (144% of basal levels on day 1). For both DOPAC and HVA, there was a decrease 2 weeks later (39.8 and 51.3%, respectively), related to their basal levels on day 1. In contrast, there were no changes in the basal concentrations of DA 1 and 2 weeks later.

Effect of HgCl₂ Infusion

The different concentrations of HgCl₂ used (40 µM, 400 µM, and 4 mM) produced increases in the striatal
FIG. 1. Basal values for DA, DOPAC, and HVA 1 day after cannula implantation, and 1 and 2 weeks later. The results are presented as means ± SEM of four or five experiments, expressed as a percentage of basal levels (100%). Significant differences: *P < 0.05 and **P < 0.01 with respect to basal level on day 1.

output of DA (Fig. 2A). The individual experiments carried out on day 1 reveal that 40 µM, 400 µM, and 4 mM HgCl₂ produced a maximum increase 75 min after the beginning of HgCl₂ perfusion (1240 ± 66%, 2500 ± 424%, and 2658 ± 337% of basal levels, respectively). Basal values recovered 120 min after administration of HgCl₂.

The highest concentrations of HgCl₂ assessed (400 µM and 4 mM) caused a significant decrease in extracellular levels of DOPAC and HVA in striatum 75 min after the beginning of HgCl₂ administration (25.6 and 29% decrease for the 400 µM concentration and 96.6 and 49.4% decrease for the 4 mM concentration with respect to basal levels, respectively) (Figs. 2B and 2C). The smallest concentration of HgCl₂ (40 µM) had no significant effect on the acidic metabolites of DA.

**Time Course of HgCl₂ Effects on DA, DOPAC, and HVA Levels**

In these experiments, rats exposed to 40 µM HgCl₂ 1 day after surgery were also exposed to 400 µM HgCl₂ 1 week later and 4 mM HgCl₂ 2 weeks later. Figure 3 presents the time course of the effects of increased concentrations of HgCl₂ on DA, DOPAC, and HVA striatal levels.

The time course was characterized by an initial phase in which DA extracellular levels increased due to the effect of 40 µM HgCl₂ (Fig. 3A). This concentration of HgCl₂ did
FIG. 3. Time course of the effects of intrastriatal perfusion of increased concentrations of HgCl$_2$ (rats exposed to 40 μM HgCl$_2$ 1 day after surgery were also exposed to 400 μM HgCl$_2$ 1 week later and 4 mM HgCl$_2$ 2 weeks later) on DA (A), DOPAC (B), and HVA (C) striatal levels. Arrow denotes the infusion of HgCl$_2$ over 60 min. The results are presented as means ± SEM of four or five experiments, expressed as a percentage of the basal levels (100%). Basal levels were considered as the mean of substance concentrations in the three samples before HgCl$_2$ perfusion. Significant differences: *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ with respect to basal levels.

not affect the basal extracellular output of DOPAC or HVA (Figs. 3B and 3C).

The second phase (1 week after the first HgCl$_2$ infusion) was characterized by an increase in DA levels 90 min after the beginning of 400 μM HgCl$_2$ perfusion (1605 ± 152% increase with respect to basal levels) (Fig. 3A). In the same period, with the same concentration of 400 μM HgCl$_2$, DOPAC and HVA levels decreased 44 ± 6% and 39 ± 1%, respectively (Figs. 3B and 3C).

The third phase (2 weeks after the first HgCl$_2$ infusion) was characterized by a small increase in DA levels 90 min after the beginning of 4 mM HgCl$_2$ perfusion (613 ± 75% of baseline) (Fig. 3A), while DOPAC and HVA levels decreased 56 ± 4% and 33 ± 6%, respectively (Figs. 3B and 3C). The decrease in DOPAC and HVA levels was not significant compared with the decrease observed after the second infusion of HgCl$_2$ (400 μM).

Intrastriatal infusion of 400 μM and 4 mM HgCl$_2$ 1 and 2 weeks after the first infusion (40 μM HgCl$_2$) produced increases in DA levels that were, respectively, 36 and 62% smaller than that observed with the first infusion. Moreover, compared with the single administrations of 400 μM and 4 mM HgCl$_2$ (Fig. 2), the administrations of 400 μM and 4 mM HgCl$_2$ to the same group of rats 1 and 2 weeks after the first HgCl$_2$ infusion (40 μM, day 1) indicated that the HgCl$_2$ effects were not only reduced but also delayed by 15 min, especially for the concentration of 4 mM (Fig. 3), even though increased concentrations of HgCl$_2$ were used in those experiments.

DISCUSSION

Previous papers reported that different administrations (chronic, acute, and intradialysate) of MeHg induced significant dose-dependent increases in the striatal DA release (Faro et al., 1997, 1998, 2000).

In this study, the administration of all concentrations of HgCl$_2$ assessed also induced significant increases in DA striatal levels. In contrast with results about DA release after MeHg intrastriatal administration (Faro et al., 2000), the effect of HgCl$_2$ administration was not dose-dependent: the increases in DA levels induced by HgCl$_2$ were the same for 400 μM and 4 mM HgCl$_2$ (∼2500%). Comparing these results with those obtained with MeHg, the increases in
extracellular DA levels induced by HgCl₂ were lower (for
the 4 mM concentration) and delayed by 30 min. It is sug-
gested that the effect of HgCl₂ reached a maximum with the
concentration of 400 μM.

The mechanism by which DA levels were increased after
intrastriatal administration of HgCl₂ could be due to a
direct action of toxicant on dopaminergic neurons at different
levels, such as membrane, vesicular release, reuptake system,
and transport systems. Previous results suggested that the
mechanism that underlies the effects of various neurotoxic
mercurials (such as Hg²⁺ and MeHg) on central synaptic
transmission differs with respect to the sites and potency of
action and reversibility (Yuan and Atchison, 1994).

The extracellular levels of DOPAC and HVA decreased
with concentrations of 400 μM and 4 mM HgCl₂, with the
decrease of striatal DOPAC being dramatic after the intras-
striatal administration of 4 mM HgCl₂. Since DOPAC is
produced intraneuronally from newly synthesized DA (Zet-
terstrom et al., 1988), the decreased DOPAC levels observed
with the concentration of 4 mM HgCl₂ can be explained as
due to a decreased DA synthesis.

The successive administrations (1 and 2 weeks later) of
incremental concentrations (400 μM and 4 mM) of HgCl₂ to
the same group of animals that received 40 μM HgCl₂
before demonstrated that the effect on striatal DA levels was
successively lower and delayed compared with the respective
effects of single administrations: 1605% versus 2583% for
the concentration of 400 μM and 613% versus 2601% for
the concentration of 4 mM. Since the levels of DA
metabolites had similar decreases after administration of
400 μM and 4 mM HgCl₂ 1 and 2 weeks later, the lower
striatal levels can be explained considering that the HgCl₂
affects the DA release mechanisms or induces neuronal
damage depending on the concentration and time of HgCl₂
administration.

CONCLUSION

The intrastriatal administration of different concentra-
tions of HgCl₂ 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 the case of the concentrations of 400 μM and
4 mM HgCl₂). It is suggested that these results may be due
to different actions for the different concentrations of HgCl₂
assessed. Thus, lower concentrations of HgCl₂ (400 μM)
may increase DA release, whereas high concentrations
(4 mM) may decrease DA synthesis (even increasing the
release of the DA previously synthesized). These results are
consistent with other results previously obtained for striatal
DA levels (Faro et al., 1997, 1998, 2000), enabling us to
undertake future studies regarding the effects of intrastriatal
HgCl₂ administration on the release of other neuro-
transmitters, such as glutamate and GABA.

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