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ARTICLE

Accumulation of Hg(II) Ions in Mouse Adrenal Gland

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Female BALBc mice were administered $HgCl_2$ at a single dose of 4 mg/kg i.p. The acute intoxication with Hg(II) salts (2 hr) caused accumulation of Hg(II) ions in the adrenal gland in general, and in the medulla, in particular. Based on data obtained with atomic absorption spectroscopy and quantitative cytochemistry, we determined the amount of mercury (II) in the adrenal glands and found it to be 14.2 ng Hg(II) (3.5 mg/kg wet weight of the adrenals). An uneven distribution of Hg(II) was found within the adrenal gland, not only between the medulla and cortex, but also within the cortex. The applied autometallographic method revealed that the cortex was negative except the zona glomerulosa, whereas the medulla showed a strong reaction localised to the chromaffin granules of the secretory cells. Both adrenaline and noradrenaline producing cells reacted. The comparison of the density of silver grains by scanning densitometry in the medulla and cortex revealed a significantly higher Hg(II) concentration in the medulla compared to the cortex (10 mg/kg vs 2 mg/kg, respectively). The results presented here suggest that there may be a connection between the symptoms of acute Hg(II) intoxication and its adrenal accumulation. (Pathology Oncology Research Vol 2, No1–2, 52–55, 1996)

Key words: Hg(II); acute intoxication; adrenal gland; atomic absorption spectroscopy, autometallography

Introduction

Although 35 years ago Cheek *et al.*[2] described that inorganic mercury can markedly increase epinephrine levels in rats, few observations have been published about the adrenal medulla of mercury intoxicated humans or experimental animals. In human mercury poisoning, the basal plasma and urinary cathecolamine levels are increased, suggesting a release from the adrenals.[5] In autopsy samples of the adrenals from individuals without any known mercury exposure, the concentration of this metal ranged from 0.1 ppm to 15.9 ppm.[6] We have not found any publication describing similar measurements in mercury intoxicated humans or experimental animals. Rasmussen and Thoracius-Ussing[10] demonstrated mercury deposition in the adrenocortical lysosomes and medullary chromaffin granules by histochemical autometallography in rats, however, without any quantitative evaluation.

Here, we studied exogeneously administered $HgCl_2$ deposition in the adrenals of BALB/c mice. They were chosen because the difference in toxokinetics between humans and mice is relatively small.[8] The results proved that with $HgCl_2$ intoxication, the main place of Hg(II)-accumulation within the adrenal gland is the medulla.

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Material and Methods

Female BALBc mice weighing 35-40 g and kept on a standard normal diet (LATI, Gödöllő, Hungary) and water ad libitum were given $HgCl_2$ dissolved in 0.5 ml 0.9% NaCl at a single dose of 4 mg/kg i.p.. Two hours later they were sacrificed and both of their adrenal glands were removed. The animals serving as controls only received 0.5 ml of solvent.

Atomic absorption spectrometry

The adrenal glands of 4 mice (2 treated and 2 control) were used to measure the amount of mercury in the organs by atomic absorption spectroscopy.[11]

Reagents

The reagents used in the determination were of p.a. grade. The water was bidistilled. The reference material solution, Mercury 1000 mg/dm^3 (FLUKA), was used to prepare calibration standards.

Samples

Mouse adrenal glands weighing 3.5-5.1 mg(wet weight) were introduced into separate teflon vessels. Nitric acid 65% p.a. 1.5 ml and H_2O_2 30% p.a. 0.5 ml was added to each vessel. MWD (microwave digestion) technology program N was applied for digestion.

Procedure

Dissolution was carried out with a MILESTONE (MLS-1200 MEGA) microwave digestion rotor. Atomic absorption spectrometry measurements of Hg were performed with a UNICAM SP1900 atomic absorption spectrometer equipped with cold vapor Hg generator and quartz window cuvette. Signals were recorded with a SEFRAN (Servotrace, Paris) PED type recorder (Servotrace, Paris) modified with the addition of a 1000 µF condensator in the integrated circuit which permitted registration of very small amount of mercury. The role of the condensator was the collection of the short-lived signals and their integration. Calibration solutions and samples were then transferred separately to the Hg generator containing 30% H_2SO_4 and 10% $SnCl_2$ solutions. The Hg formed was driven out with an air flow at 1 l/min through $Mg(ClO_4)_2$ into the quartz cuvette. The Hg signal was measured at 253.7 nm. Quadratic regression was calculated to define the calibration curve.

Light microscopy

For light microscopic visualization of Hg(II) ions, the autometallographic method of Danscher and Schröder[3] was carried out. The adrenal glands were fixed in 10% neutral formalin and embedded in paraffin. Sections (4-6 µm thick) were placed on slides, deparaffinized and incubated with developer for 45 min. The developer was always freshly prepared containing a saturated aquaeous solution of gum arabic (60 ml), citrate buffer (22.5 g citric acid, $1H_2O$ and 23.5 g sodium citrate, $2H_2O$/100 ml bidistilled water) (10 ml), hydroquinone (5.67 g/100 ml water) (30 ml), $AgNO_3$ solution (8.5 g/50 ml) (0.5 ml). Subsequently the slides were rinsed in distilled water, dehydrated and mounted with Canada balsam. The amount of silver grains in the cortex and medulla was estimated by a densitometric evaluation of the microphotograph taken of the sections (LaborMIM densitometer, Hungary).

Determination of the relative volumes of the adrenal cortex and medulla

Twenty-five µm thick freeze-dryed serial cryostate sections were prepared that covered the whole extension of the adrenal gland. The volume of the cortex and medulla was estimated by making photo prints of each section and cutting out separately the cortex and the medulla. After measuring the respective weight with an analytical balance and assuming the same thickness for all sections we calculated the medulla to cortex volume ratio.

Electronmicroscopy

The adrenal glands from untreated and Hg(II) treated animals were fixed by immersion into phosphate buffered 3% glutaraldehyde at 4°C overnight. After rinsing the tissue in physiological saline, 40 µm frozen sections were prepared. The unmounted sections were subjected to silver amplification as described above, rinsed in distilled water, osmicated, alcohol dehydrated, contrasted with uranyl acetate and embedded into Araldite (FLUKA). Subsequently, thin sections were made and investigated in a TESLA BS500 electronmicroscope without any further counterstaining.

Results

Atomic absorption spectrometry

Hg (II) was readily detectable in the adrenal glands of the 2 hr-intoxicated mice. Data points and the calibration curve are shown in *Fig. 1*. In contrast, no mercury was found in the adrenals of the control animals, the corresponding values remaining as low as those for the reagent controls (data not shown).

In the Hg(II)-treated animals, the detected values – 11.87 and 16.56 ng – with an average of 14.2 correspond to the Hg(II) concentration of 3.4 and 3.6 mg/kg (in case of 3.5 and 4.6 mg adrenals) approaching the concentration given (4 mg/kg).

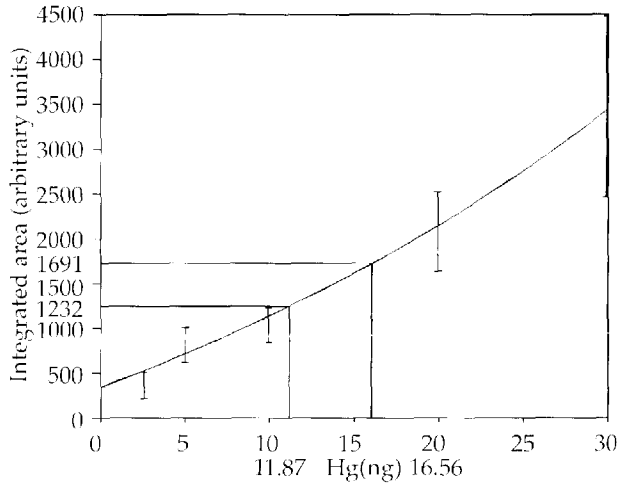

Figure 1. *Hg(II) content of mouse adrenal glands determined by atomic absorption spectrometry, 2 hr after the administration of $HgCl_2$ at a dose of 4 mg/kg i.p. No detectable amount of Hg(II) was found in the controls. Experimental data (x) are in ng. Calibration curve: ng±SD; n=5.*

Figure 2. Autometallographic staining for Hg(II) in the adrenal glands according to (3). Light microscopic picture of $HgCl_2$ treated (A) and untreated, control (B) mouse adrenal gland (c: cortex, m: medulla). The medulla and the zona glomerulosa show positive reaction (A) but no specific reaction product is seen in controls (B). Scale bar: 100 μm.

Light microscopy

The distribution of the incorporated Hg(II) displayed a characteristic pattern; the medulla contained much higher number of silver grains than the cortex *(Fig.2a)*. In the cortex, the reaction was localized to the cells of the zona glomerulosa with only background level seen in the zona fasciculata and reticularis *(Fig.2a)*. Scanning densitometry of silver grains further supported the suggestion that the Hg(II) concentration in the medulla and cortex was significantly different *(Fig.3)*. The comparison of the density of silver grains measured by the territories under the densitometric curve in the medulla and cortex revealed a fivefold difference in Hg(II) concentration in the respective parts of the gland *(Fig.3)*. The weight measurements of the cortex and medulla photoprint cut-outs showed that the cortex/medulla volume ratio was 5:1. Thus the higher amount of Hg(II) in the medulla is even more significant and indicates a specific accumulation.

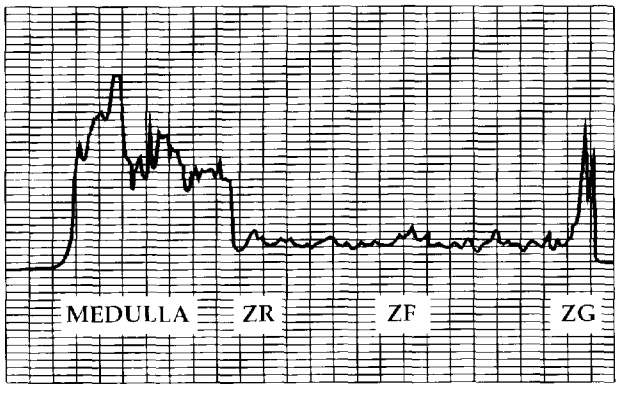

Figure 3. Scanning densitometry of the autometallographic sections of the adrenal gland intoxicated with Hg(II). Note the two peaks at the medulla and zona glomerulosa.

Electron microscopy

Mercury-induced silver deposits were mainly found in the chromaffin granules of the medullary endocrine cells *(Fig.4a)*. Interestingly, the deposits appeared both in the adrenalin and noradrenalin producing cells, but only in some of the secretory granules. The majority of these were free from reaction product *(Fig.4a)*, even though the number and size of the silver deposits increased with the incubation time of the developer *(Fig.4b)*. Some of the lysosomes of the chromaffin cells also contained silver grains, similar to the cells in the cortex of the zona glomerulosa (data not shown).

Discussion

The importance of the investigation of mercury intoxication is underlined by the continuing human exposure in a wide range of professions to metallic Hg as well as Hg(II) compounds. In the present study, we demonstrated that acute intoxication (2 hr) with Hg(II) salts induces accumulation of Hg(II) ions in the adrenal gland in general, and in the medulla, in particular. Based on data obtained with atomic absorption spectroscopy on the one hand, and in situ cytochemistry on the other hand, we calculated a 10 mg/kg concentration of Hg(II) in the medulla, when the total single dose administered was 4 mg/kg, suggesting accumulation. As for mercury-exposed human beings, no relevant data have been found for making any comparison. We used autometallography to investigate the localization of mercury in the adrenals. The application of this method is especially useful for the detection of inorganic mercury.[7] A characteristic uneven distribution of Hg(II) within the adrenal gland was found after the 2 hr treatment. Hg(II) concentration evaluated by scanning densitometry was significantly higher in the medulla than in the cortex. This observation is consistent with the report of Rasmussen and Thoracius-Ussing[10] detecting Hg(II) in some of the chromaffin granules of medullary cells as well as in the lysosomes of zona glomerulosa after a one week methyl-mercury treatment. Danscher *et al*[4] described Hg(II) accumulation in the adrenal medulla of primates with amalgam dental fillings. Although these authors observed the Hg(II) in the lysosomes, the fact that mercury intoxication also elicits cathecolamine release[5] suggests that chromaffin granules in medullary cells may be affected by Hg(II), as shown in this study as well as in that of Rasmussen and Thoracius-Ussing.[10] It is noteworthy that phaeochromocytoma is also characterized by elevated cathecolamine levels, therefore this finding in the case of a subtle form of Hg(II) intoxication may be misleading. Hg(II) was also present in the zona glomerulosa after the 2 hr intoxication but was absent in the zona fasciculata and reticularis. In light of the results of Rasmussen and Thoracius-Ussing[10] it seems likely that, with prolonged $HgCl_2$ treatment, this metal may appear in these two layers of the

cortex, too. The incorporation of Hg(II) into the lysosomes of the cells in the zona fasciculata and reticularis induced discernible vacuolization and necrosis after a long-term methyl-mercury treatment as well as functional changes,[13] including 21-alpha-hydroxylase activity.

There are open questions as to the mechanism of action of Hg(II) ions in the living cells specified above. Although the most prominent candidates for Hg(II) binding are the molecules with SH groups, phosphate and carboxyl groups also display affinity to mercury ions.[7] According to Brown and Smith,[1] Hg(II) binds to the lipid membranes, which may account for at least part of the functional changes induced by Hg(II) intoxication. Nevertheless, morphological studies, including the presented one, repeatedly proved Hg(II) localization in the matrix of the lysosomes as well as secretory granules.[3]

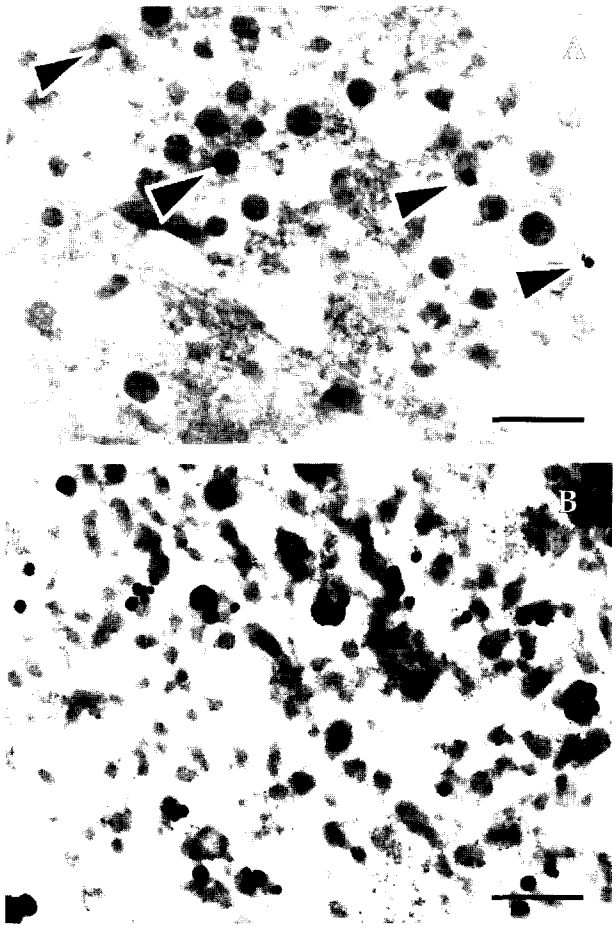

Figure 4. Electron microscopy of the Hg(II) in chromaffine granules of the adrenal cells after autometallographic reaction. Intragranular single silver grains are shown (A), which become more prominent as the reaction proceeds (B). Some chromaffin granules contain more than one small silver grain, while in others the grains are larger, coalesced and extend beyond the limiting membrane of the granules (B). No counter staining. Scale bar: 0.5 μm.

There may be two mechanisms whereby mercury(II) appears in lysosomes. Either it binds directly to lysosomal enzymes abundant in SH groups, or proteins loaded with mercury become internalized by microautophagy.

The accumulation of Hg(II) in the chromaffin granules of medullary cells may also be attributed to the presence of sulfur-containing molecules like chromogranin and several neuropeptides in these granules.[12,14] Further studies are required to investigate impaired cell functions caused by Hg(II) accumulation.

Acknowledgement

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