EFFECTS OF INORGANIC AND ORGANIC MERCURY ON CORTICAL AND HIPPOCAMPAL ACTIVITY IN RATS

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ABSTRACT: People are exposed to inorganic or organic mercury in various occupational and environmental settings. The aim of this study was to see the effect of an inorganic and an organic mercury compound on spontaneous and stimulus-evoked central nervous activity in rats under identical experimental conditions. Male Wistar rats were treated for 10 weeks with 0.5 and 2.0 mg/kg b.w. of mercuric chloride ($HgCl_2$) or methylmercury chloride (CH_3HgCl) per os by gavage. For neurophysiological investigation, the animals were anaesthetised, placed in a stereotaxic instrument, and the left hemisphere was exposed. Activity was recorded from the primary somatosensory, visual and auditory fields, and the hippocampal CA1 region. There was no qualitative difference between the effects of inorganic and organic mercury but the same dose of methylmercury generally elicited more marked alterations. In the spontaneous cortical and hippocampal activity, the peak of activity was shifted to higher frequencies. The duration of the somatosensory and visual cortical evoked potentials were significantly shortened by organic but hardly affected by inorganic mercury. Methylmercury had a stronger effect on the tetanic potentiation of hippocampal evoked activity (population spike) than mercury chloride had. Our results indicate that differences in chemical character and neurotoxicity are only partially in line.

KEY WORDS: Inorganic mercury, organic mercury, cortex, hippocampus, rat, neurotoxicity

INTRODUCTION

Under natural conditions, the bioavailability of mercury is minimal. With the beginning of human technology, however, mercury exposure from various sources has been present in large populations. In occupational exposure, both elementary mercury and inorganic mercury salts (Kark, 1994) as well as alkyl and aryl mercury compounds (Key et al., 1977) are present. In the exposure of the population, mercury released into the environment and dental amalgam are the major sources (Trepka et al., 1997). There is an extensive biogenic interconversion between inorganic and organic mercury in the environment (ATSDR, 1999), so that both must be taken into account when investigating toxic effects.

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Exposure by various forms of mercury results in different absorption and tissue distribution patterns. The Hg^{2+} ions of mercuric chloride are bound to proteins so that the intestinal absorption is only ca. 20 % (Nielsen, 1992) and the amount absorbed can slowly cross the blood-brain barrier (Aschner and Aschner, 1990). The lipophylic alkyl mercury compounds are absorbed better and transported unchanged into the central nervous system (Aschner and Aschner, 1990). There, a partial dealkylation (Gallagher and Lee, 1980; Friberg and Mottet, 1989) happens so that the inorganic form of Hg deposited in the brain bound to sulfhydryl ligands, cortex and thalamus being preferential sites (Möller-Masden, 1990).

Both inorganic and organic forms of mercury can cause various neurological disorders. In humans, paraesthesia, changes of personality and memory loss were observed (Langauer-Lewowiczka et al., 1986; Clarkson, 1989). A number of neurological disorders were seen in persons occupationally exposed to mercury. Abnormal EEG was seen in workers exposed to mercury vapor (Piikivi and Tolonen, 1989) and in subjects after intrauterine methylmercury exposure (Eto et al., 1992). Latency changes of brainstem auditory evoked potential were found to correlate with body load by both metal and organic mercury (Counter et al., 1998).

In our earlier studies, alterations in the spontaneous (Dési et al., 1996; Nagymajtényi et al., 2000) and stimulus-evoked (Schulz et al., 1997; Papp et al., 2000) cortical activity were seen on subchronic $HgCl_2$ treatment in rats. In this study, our aim was to reveal the effects of an inorganic ($HgCl_2$) and an organic (CH_3HgCl) mercury compound on the spontaneous and stimulus-evoked central nervous electrical activity in rats under identical experimental conditions.

MATERIALS AND METHODS

The experiments were performed on Wistar rats (young adult males, ca. 250 g body weight, 10 animals per group). The animals, kept under conventional conditions (up to five rats/cage, 22 ± 2 °C, 60 ± 10% humidity, 12 hours light-dark cycle with light starting at 6.00 a.m., rodent food and water *ad libitum*) were treated with 0.5 and 2.0 mg/kg b.w. mercuric chloride ($HgCl_2$; analytical grade, Reanal, Hungary) or methylmercury chloride (CH_3HgCl; analytical grade, Aldrich) per os by gavage, daily for 10 weeks. Both substances were dissolved in distilled water to 1 ml/kg administration volume. Control animals received distilled water.

Immediately following the 10-week period, the animals were anaesthetized with 1000 mg/kg urethane, i.p., placed in a stereotaxic instrument, and the left hemisphere was exposed. Silver electrodes were placed on the primary somatosensory, visual and auditory areas. For recording hippocampal population spikes (Anderson et al., 1971), a bipolar steel needle electrode was inserted into the left peforant path (stereotaxic coordinates: AP -6, L 4.5, V 4; Paxinos and Watson, 1982) and a unipolar steel recording electrode into the hippocampal CA1 region (AP -3, L 2, V 2 to 3). The animals were covered with a warm cloth and were put aside for 30 min for recovery. Then, electrocorticogram (ECoG) was recorded from the three primary sensory areas, together with the spontaneous hippocampal activity, simultaneously

for 15 minutes. From the records, the relative spectral power of the frequency bands was determined and the ratio of the spectral power in the slow (delta + theta) vs. fast ($beta_1$ + $beta_2$) bands (ECoG index; Dési, 1983) was calculated. Then, cortical evoked potentials were recorded. Somatosensory stimulation was done by a pair of needles inserted into the nasal part of skin, in the area of the whiskers, delivering rectangular electrical stimuli (1 Hz, 3-4 V, 0.2 ms). Visual stimulation was performed by flashes (1 Hz, 60 lux) conducted from a flash generator via an optical fiber directly into the contralateral eye of the rat. For acoustic stimulation, clicks (1 Hz, 40 dB), from a small earphone were applied into the contralateral ear of the rat. A single train of 50 stimuli for each modality per rat was applied and the evoked activity recorded. Hippocampal population spikes were elicited by stimulation of the perforant path and recorded from CA1 (see above), using a train of 20 stimuli at 0.3 Hz frequency, repeated every 20 min. After averaging, latency and duration of the main waves were manually measured. All recordings and off-line analysis were performed by a PC using the NEUROSYS 1.11 software (Experimetria Ltd., U.K.).

The normality of the electrophysiological results was tested by the Kolmogorov test. Then, one-way ANOVA was used to find significant ($p<0.05$) alterations.

RESULTS

In the spontaneous cortical and hippocampal activity, both mercury compounds caused decreased activity in the range of lower and increased activity in that of higher frequencies (*Fig. 1*). The effect was the most characteristic and significant ($p<0.05$) in the visual cortex and in the hippocampal CA1 area. The changes in the other areas were similar but less strong (and, due to higher variability animal-to-animal, did not reach the level of significance). A clear dose dependence was not seen in every case but the general trend of activity shifted to higher frequencies was always visible.

The largest difference between the effects of organic and inorganic mercury was seen in the cortical evoked activity. Methylmercury treatment caused a slight increase in the latency of the evoked potentials while their duration was decreased (*Fig. 2*). The latter was significant in the somatosensory and visual evoked potential but minimal in the auditory one. The effect of mercuric chloride was equivocal.

In case of hippocampal population spikes, the effect of mercury on the so-called tetanic potentiation was investigated. A brief tetanizing series of stimuli (ca. 50 Hz, 3 sec) induces an increase of the population spike amplitude (Bliss and Lomo, 1973). It was found in both treated groups that the increase of the amplitude was less than that in the controls (*Fig. 3*). The effect of methylmercury was somewhat stronger than that of mercuric chloride.

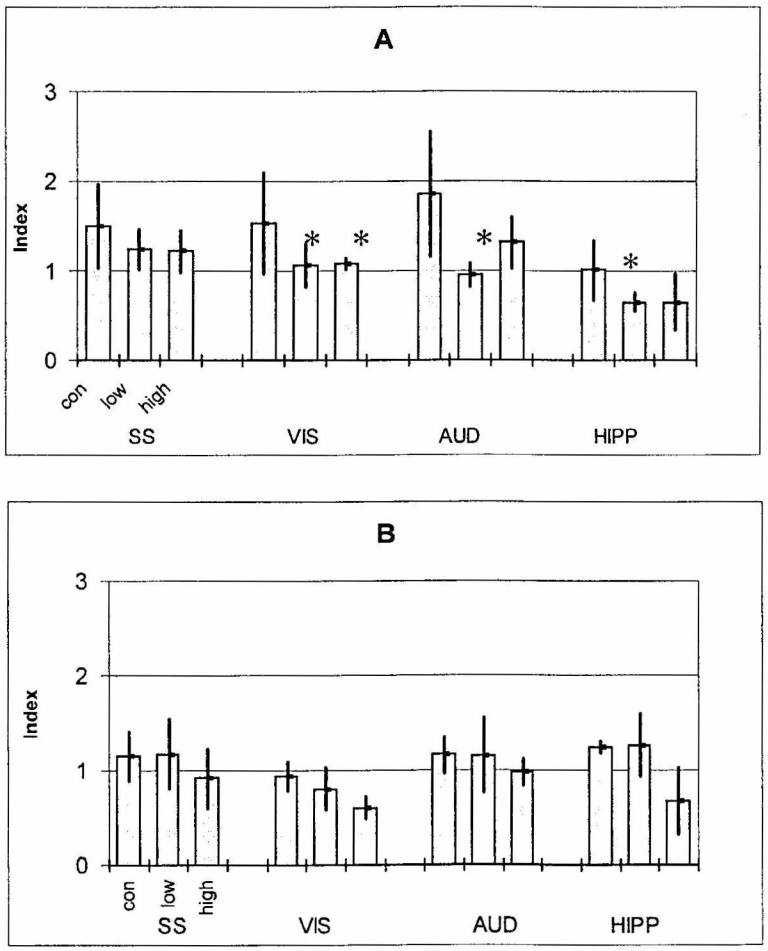

*Fig. 1. Effect of methylmercury (A) and mercuric chloride (B) on the cortical and hippocampal spontaneous activity, represented by the ECoG index (ordinate). SS: somatsensory cortex; VIS: visual cortex; AUD: auditory cortex; HIPP: hippocampal CA1 area. Con: control; low: 0.5 mg/kg; high: 2.0 mg/kg. Error bars: S.D; *: $p<0.05$*

DISCUSSION

The nervous system is the target of numerous environmental pollutants. Some of them, like insecticide agents, are designed to cause death by nervous dysfunction (Nagymajtényi et al., 1988, 1994; Dési and Nagymajtényi, 1999). Others, like most of the heavy metals, may or may not have a practical biocide activity but have been emitted in large quantities into the environment.

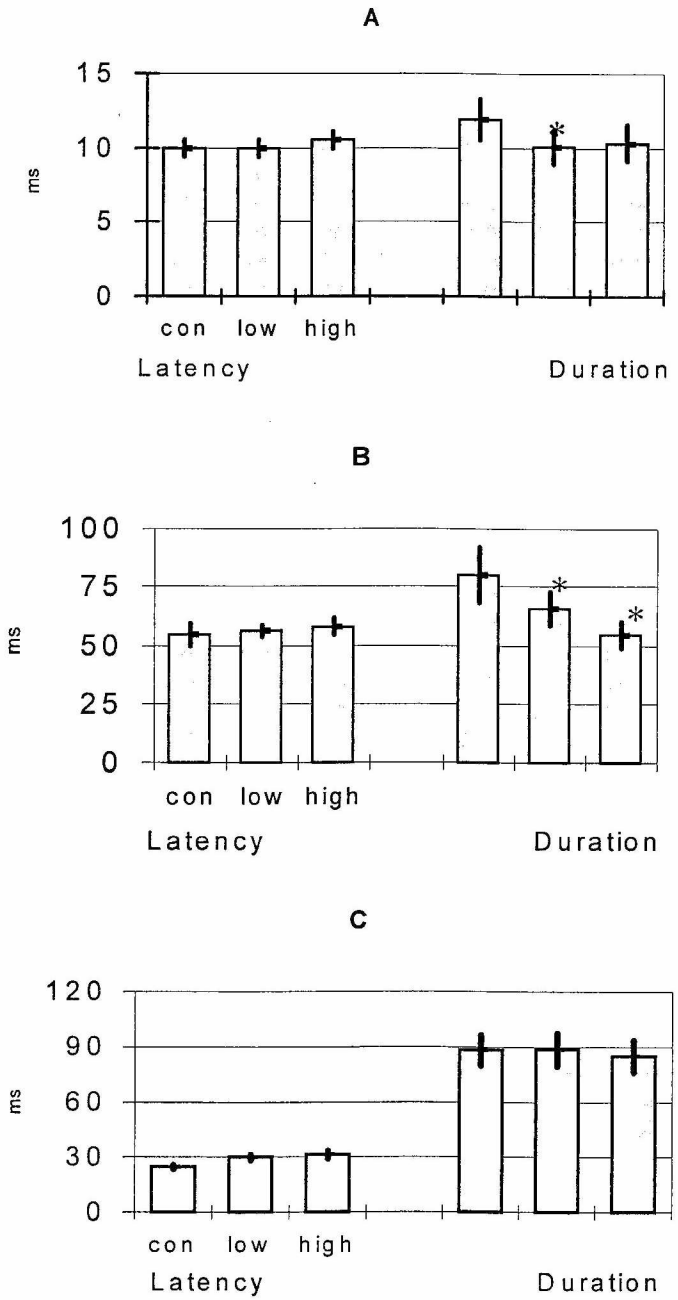

Fig. 2. Effects of methylmercury on the latency and duration of cortical sensory evoked potentials (A: somatosensory; B: visual; C: auditory). Further details as in Fig. 1.

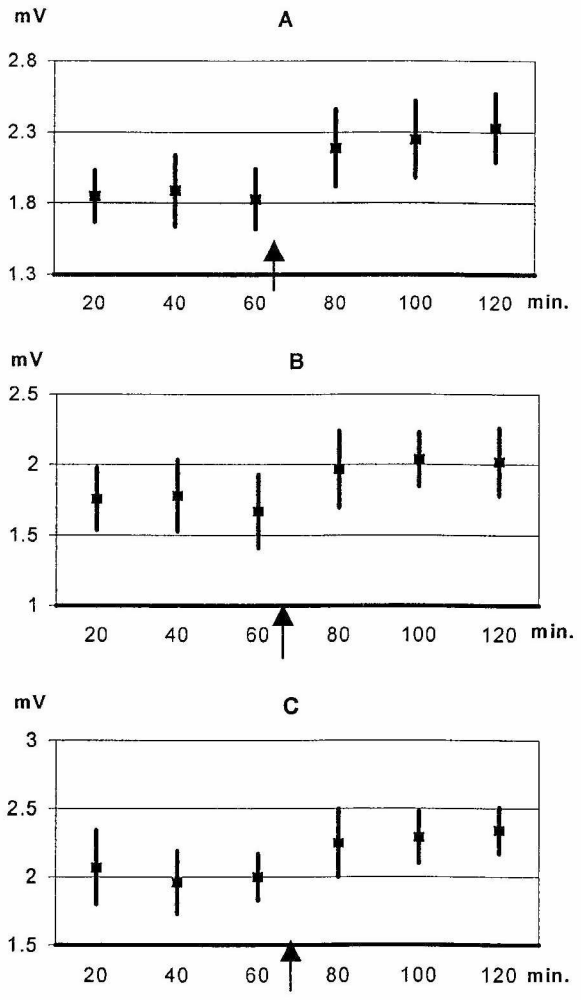

Fig. 3. Tetanic potentiation of the population spike amplitude in control (A), $HgCl_2$-treated (B) and met-Hg–treated (C) animals. Abscissa: time from beginning of recording. Ordinate: population spike amplitude. Tetanic potentiation was given between the 60^{th} and 80^{th} minute (arrow). Mean values ± S.D.

In the present study, inorganic and organic mercury was seen to disturb the function of the rats' central nervous system. By treating rats with similar doses of methylmercury, Coccini et al. (2000) found a strong effect on muscarinic cholinergic receptors, especially in the hippocampus. This supports our findings, as the cholinergic system is known to play a major role in modulation of the cortical (Metherate et al., 1992) and hippocampal (Benardo and Prince, 1982) activity. Increased spontaneous activity, in neocortical and other areas, is usually concomitant with decreased stimulus-evoked or phase-bound activity. In the neocortex, this was demon-

strated in the present experiment and in on previous studies of ours (Schulz et al., 1997; Nagymajtényi et al., 2000).

In our experiments, the difference between the effects of mercuric chloride and methylmercury was in most cases slight. This is in contrast with the known human consequences of exposure to either of them and also with certain published findings in animals, but can possibly be explained with the fact that the form of mercury deposited in the brain tissue is Hg^{2+} bound to sulfhydryl groups in both cases (Gallagher and Lee, 1980; Möller-Masden, 1990).

From past and present emission, mercury is present in the environment and, hence, in various items of food. To recognize and prevent serious human consequences, more needs to be learned about its mechanism of toxicity including effects on the nervous system.

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