

NEUROPHYSIOLOGICAL CHANGES CAUSED BY COMBINED TREATMENT WITH HEAVY METALS AND ETHANOL IN RATS

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ABSTRACT: In our days the whole population, especially in the developed countries, is continuously exposed to various environmental pollutants, e.g. heavy metals, and/or to ethanol in case of alcohol addicts. As it was shown in previous experiments, these chemicals can cause considerable changes in certain sensitive functional parameters of the central nervous system. Since the population is usually exposed to several xenobiotics simultaneously, the aim of this study was to investigate certain functional neurophysiological parameters following subchronic combined treatment with well-known neurotoxic compounds. Rats were treated daily by gavage with low doses of lead and mercury, given alone or in combination with 5% ethanol in the drinking water, in different periods of intra- and/or extrauterine development of the nervous system. The results showed that the combined application of the above neurotoxic substances induced more marked alterations in the investigated neurophysiological parameters (electrocorticogram, cortical evoked potential) than if they were given alone. These results emphasize the necessity of further analysis of the combined effects induced by environmental xenobiotics – not only in animal experiments but also in exposed human populations.

KEY WORDS: Lead, mercury, ethanol, electrocorticogram, cortical evoked potential, rat

INTRODUCTION

It is well known that the health of the population is continuously influenced by various environmental pollutants, such as heavy metals, originating from combustion of fossil fuels and from industry. At the same time there is an established, but in some regions increasing, number of addicts to alcohol, being also a neurotoxic compound. The chronic low-level exposure to these chemicals represents an important risk especially for more susceptible subpopulations, such as pregnant women, newborn and breast fed babies (WHO, 1989; 1991).

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Acute and chronic intoxication caused by the aforementioned compounds, including the nervous system pathology, were studied by several authors (Lille et al., 1994; Winneke et al., 1994; Winneke, 1995). There is, however, only a small number of data in the literature about the neurofunctional alterations induced by their simultaneous application, particularly as for pre- and/or postnatal intake (Flora et al., 1999).

In a previous study, it was found that administration of low-level doses of lead and mercury, given in different periods of the intra- and extrauterine development, caused changes of certain spontaneous and evoked activity forms of the nervous system (Nagymajtényi et al., 1998; 2000). The aim of the present study was to investigate the electrophysiological changes caused by pre- and postnatal low dose lead and mercury treatment in combination with ethanol.

MATERIALS AND METHODS

Female Wistar rats (10 animals/group) were orally treated by gavage with 80.0 and 320.0 mg/kg lead ($C_4H_6O_4Pb \cdot 3H_2O$), 0.4 and 1.6 mg/kg mercury ($HgCl_2$), 5% ethanol in drinking water, each given alone or in a metal + ethanol combination; from the 5th to 15th day of pregnancy (P protocol), or in the same period of pregnancy + for 4 weeks of lactation (P + L protocol), or in the mentioned periods of pregnancy and lactation + for further 8 weeks after weaning in case of male offspring (F1 generation) (P + L + P protocol). The control rats were given only saline, orally.

The neurophysiological parameters of F1 male rats were investigated at the age of 12 weeks. The anesthetized rats (1000 mg/kg urethane ip.; Bowman and Rand, 1980) were placed in a stereotaxic instrument. After opening the skull, silver electrodes were directly placed on the primary somatosensory, visual and auditory foci (Zilles, 1982). The electrocorticogram (ECoG) was simultaneously recorded from the three cortical areas for 15 minutes; the analyzed parameters were the mean frequency and the power spectrum of the frequency bands.

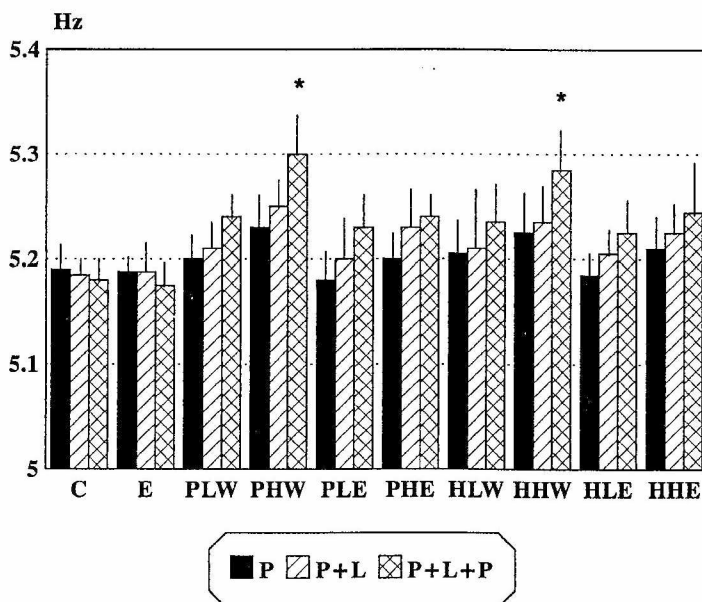
Sensory evoked potentials were recorded with the same electrodes from the primary somatosensory, visual and auditory foci. Somatosensory stimulation (1 Hz, 3–4 V, 0.2 ms) was performed by electrodes pricked into the whiskery part of skin; the visual stimuli were flashes (1 Hz, 60 lux) conducted via glass fibers directly to the eye of the rat; for auditory stimulation clicks (1 Hz, 40 dB) of a small earphone put into the ear of the animal were used. Of each modality, 50–50 evoked potentials were averaged, and the latency, duration, and amplitude of the averaged potential were measured off line.

The experiments were approved by the Ethical Committee for the Protection of Animals in Research of the University.

The data were checked for normality by means of the Kolmogorov–Smirnov test and were analyzed by univariate ANOVA. Post hoc analysis of group differences was performed by a subsequent LSD test setting the probability level at $p < 0.05$.

RESULTS

The investigated parameters of ECoGs showed dose- and treatment protocol-dependent changes in all treated groups. Compared to the control, lead and mercury caused, in the P or P + L protocols, only a slight increase of the mean frequency, while in the P + L + P groups the change induced by the metals was significant. Ethanol administration decreased the mean frequency in P + L + P variations (*Fig. 1*). Compared to the control, increased mean frequency was seen in the lead + ethanol and mercury + ethanol treatment groups, but these changes were less expressed than in case of administration of the metals alone, especially in the high dose P + L + P groups. In the background of these data, there was a lowered activity of the slow frequency bands caused by the metals while the activity in the faster bands was increased. The visual and auditory ECoGs showed similar tendencies in the treated groups.



*Fig. 1. Changes in mean frequency of somatosensory ECoG (C: control; E: ethanol; PLW/PHW: lead, low/high dose with water; PLE/PHE: lead, low/high dose with ethanol; HLW/HHW: mercury, low/high dose with water; MLE/MHE: mercury, low/high dose with ethanol); * $p < 0.05$.*

The latency and duration of the cortical evoked potentials were also changed by the metal or metal + ethanol combined administration in a dose- and treatment protocol-dependent way. Lengthening of the latency of somatosensory evoked potentials caused by sole administration of lead, mercury, or ethanol was, compared to the control, similar and rather slight in the P and P + L protocols, but in the high dose P + L + P groups a significant alteration was seen (*Fig. 2*). Even longer laten-

cies were seen in the groups with simultaneous metal and ethanol treatment, especially in the higher dose P + L + P groups. Compared to the data of the metal-only groups, however, the further lengthening of the latency of evoked potentials caused by the combinations was not significant. The latency change of the visual and auditory evoked potentials was entirely analogous.

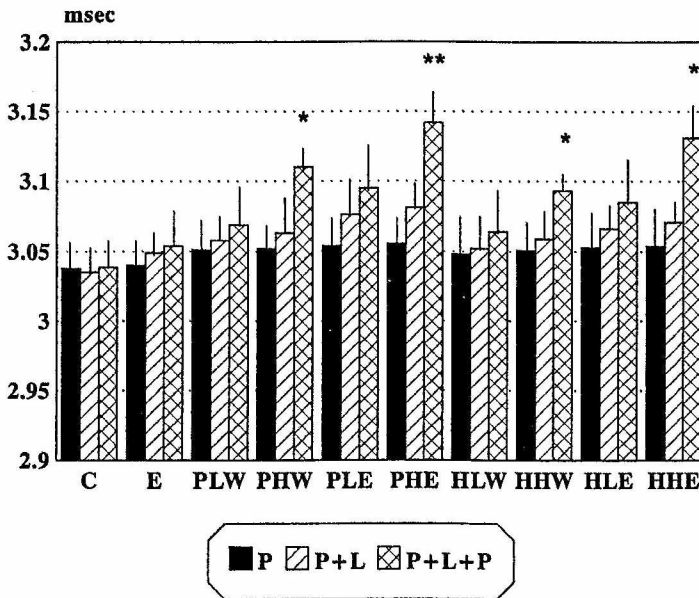


Fig. 2. Changes of somatosensory latency (C: control; E: ethanol; PLW/PHW: lead low/high dose with water; PLE/PHE: lead low/high dose with ethanol; HLW/HHW: mercury low/high dose with water; MLE/MHE: mercury low/high dose with ethanol); * $p < 0.05$, ** $p < 0.01$.

DISCUSSION

It was observed in both human and animal investigations that pre- and postnatal inorganic lead and mercury exposure can cause nervous system disorders because they can pass the placental and blood-brain barriers (Aschner and Aschner, 1990; Goyer, 1990; Warfringe et al., 1992; Plockinger et al., 1993). It was also described that newborn babies can be exposed for several months via metal-containing breast milk (Schramel et al., 1988; Klein et al., 1994; Oskarsson et al., 1995; 1996). Ethanol, being an organic solvent, also crosses the placental barrier, and can thus disturb the intra- and extrauterine development of the nervous system (Stressguth et al., 1986; Cortese et al., 1997).

The changes of the ECoGs (increased frequency, redistribution of wave band activity), and evoked potentials (latency lengthening, decrease of amplitude) are in

accordance with the effects of the one-by-one or combined administration of the metals and ethanol. In case of electrocorticograms, the toxic effects of lead or mercury with ethanol seemed to be less marked, while the changes in the evoked potentials were more considerable. In both cases, the simultaneous administration of a metal and ethanol affected the investigated processes in an additive way; their trends, however, had opposite directions.

The results of our study point to the occurrence of a possible toxicological process. Although the changes of the investigated spontaneous and evoked activities in the P and P + L groups treated by lead, mercury, ethanol, or their combinations were not significant, their tendency clearly showed a functional alteration caused by the compounds. Considering that these parameters were recorded at the end of the 12th postnatal week when, in the P and P + L protocols, the animals had received no more treatment for 12 or 8 weeks, the low-level inorganic metal, ethanol, or combined exposures seem to have considerable harmful potential during the early pre- and postnatal development of the nervous system. The post-weaning exposure, of course, increased this neurotoxic effect, and as a consequence, the functional changes became more marked and reached statistical significance.

A complex mechanism can be supposed as an explanation of the phenomena presented. Data of animal studies showed that pre- and/or postnatal lead and mercury can disturb the neurotransmitter systems (cholinergic, adrenergic, GABAergic etc.) and also act on the ion channels affecting this way the transmembrane ionic currents of the neurons (Hare et al., 1990; Audesirk and Audesirk, 1991; Lasley, 1992; Bielarczyk et al., 1994; Struzynska and Rafalowska, 1994; Sirois and Atchinson, 1996; Zwart et al., 1997; Braga et al., 1999). Ethanol also has an influence on neurotransmitters and it causes pathomorphological processes with functional consequences (Arendt et al., 1988; Moore et al., 1997). The changes of spontaneous activity can be the result of altered activity of the neurotransmitters affected by the metals, ethanol, and especially by their combinations. The lengthened latencies of evoked potentials could, in turn, be the combined consequence of the disturbed transmitter mechanisms and also the altered ion channel functions.

It is well known that a direct transfer of the data from animal experiments to man is seldom correct. However, considering the higher susceptibility of the developing nervous system in the pre- and early postnatal stages to xenobiotics, including heavy metals and ethanol, it can be stated that a simultaneous, low-level pre- and postnatal exposure of the population to these neurotoxic compounds should be regarded as a real functional-neurotoxic risk, especially for the exposed women's fetuses and suckling babies. Thus, it seems to be important to follow the changes of certain, sufficiently sensitive, neurophysiological markers in the population in order to obtain correct information about the consequences of a combined low-level exposure by neurotoxic compounds.

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