Dynamics of Central and Peripheral Evoked Electrical Activity in the Nervous System of Rats Exposed to Xenobiotics

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ABSTRACT: The complex chemical pollution of our environment results in mass human exposure to a number of proved or supposed neurotoxicants. In most cases, there are no overt symptoms of nervous system damage, thus new sensitive indicators of the resulting functional alterations still need to be worked out. Various forms of evoked electrical activity of the central and peripheral nervous system are routinely recorded in experimental animals and in humans, and are known to be sensitive to damaging influences. In the present work, male Wistar rats (300–350 g body weight) were acutely or subchronically treated with various neurotoxic heavy metals and dynamic alterations in different forms of evoked activity (somatosensory cortical evoked potentials and peripheral nerve action potential) were observed.

A series of 50 stimuli was applied and the first and last five evoked responses were averaged. The changes in the amplitude and latency of the responses over the series (last 5 vs. first 5) and the dependence of this difference on the frequency of stimulation were calculated. It was found that several of these variables, e.g., the amplitude of the cortical evoked response, were sensitive and dose-dependent indicators of the nervous system damage caused by the heavy metal exposure. On the basis of our results, new, easy to use functional tests for detection and follow-up of nervous system damage of environmental origin could be developed.

KEY WORDS: Cortical evoked potential, nerve action potential, heavy metals, rat

INTRODUCTION

The role of the environment alconditions in the causation of a number of chronic diseases is being more and more recognized. Xenobiotics, entering the human body by food or water ingestion and from the atmosphere, are a major source of exposure leading to chronic illness, including damages to the nervous system. Several environmental compounds – among those, heavy metals – are known to be neurotoxic, little is known, however, about their possible population effects at low doses and long exposure times. Heavy metals, such as lead, mercury, or manganese, have important uses in industry and agriculture and are major constituents of hazardous waste,

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so their possible role in influencing the population's health state deserves special attention.

Two main sources of environmental lead exposure (ATSDR, 1999a) have been leaded petrol, and lead paints in buildings. In humans, childhood chronic inorganic lead exposure resulted in lowered IQ and learning difficulties in children (Bellinger et al., 1989; Needleman and Gatsonis, 1990). Otto et al. (1985) observed character-istic EEG and auditory evoked potential alterations in schoolchildren after several years of exposition to lead. In rats, postnatal exposure to Pb^{2+} caused EEG disorders and learning disability (Kumar and Desiraju, 1992; Nagymajtényi et al., 1998). Alterations of sensory evoked potentials in rats treated subchronically with oral lead acetate were described by Nagymajtényi et al. (1997).

Mercury (ATSDR, 1999b) is another major metal toxicant with partly different consequences of exposure to its inorganic vs. organic forms (Yuan and Atchison, 1994). Human occupational exposure to metal or inorganic Hg caused alterations of the cortical activity (EEG: Piikivi and Tolonen, 1989; sensory evoked potentials: Counter et al., 1998). In our previous experiments, subchronic oral treatment with $HgCl_2$ caused alterations in the spontaneous (Dési et al., 1996) and evoked (Schulz et al., 1997; Papp et al., 2000) cortical activity.

Manganese is, in sharp contrast to lead and mercury, an essential microelement (ATSDR, 2000). High environmental levels, resulting from the use of a Mn-containing petrol additive (Lynam et al., 1999), agricultural fungicides (Ferraz et al., 1988), and alkali-manganese dry cells, can possibly result in toxic mass exposure. The brain is among the primary target organs in chronic manganese exposure (Roels et al., 1987), where excess Mn can be deposited (Mena et al., 1967). Compulsive and aberrant behavior, emotional instability and hallucinations are typically found in manganese-induced central nervous damage. In animals, manganese was found to block voltage-dependent Ca-channels (Büsselberg, 1995). The release of several transmitters was reduced by Mn (Takeda et al., 2003).

In case of low-level environmental burden, as it usually is the case with the population, the known metal-specific alterations in various forms of nervous system activity are usually not manifest enough to allow detection of either the exposure or the resulting health effect. In the present study, our aim was to see if a "dynamic feature", amplitude and latency change during a stimulus series, of the central and peripheral evoked activity in the nervous system of rats, exposed experimentally by lead, mercury and manganese, react sensitively to the exposure. A further aim was to try to localize these effects spatially, within the involved parts of the nervous system, and temporally, in terms of time needed for their appearance.

METHODS

Young adult male Wistar rats, kept under conventional conditions, were used. In case of subacute exposure, the animals received doses of 300 mg/kg Pb^{2+} (Pb-acetate), 2.0 mg/kg Hg^{2+} ($HgCl_2$), or 17 mg/kg Mn^{2+} ($MnCl_2$) 5 times a week, for 10 weeks. In acute exposure, higher doses (1000 mg/kg Pb^{2+}, 7.0 mg/kg Hg^{2+}, or

50 mg/kg Mn^{2+}) were given once. Controls received distilled water. For recording (at the end of the treatment period in subacutely treated animals, and before administration of the heavy metal in acute treatment) the animals were anesthetized with 1000 mg/kg urethane, the head was fixed, and the left hemisphere exposed. Somatosensory cortical evoked potentials were recorded from the primary focus. The stimulus was an electric square pulse (3-4 V, 0.05 ms, 1 Hz) delivered to the right whisker pad. Stimulation was performed in series of 50 pulses. From the animal's tail, compound action potential of the tail nerve was lead off by delivering electric pulses (2-3 V, 0.05 ms, 1 to 50 Hz) via one pair of needle electrodes and recording the potential via another. From subacutely treated animals, all activity forms were recorded once and group comparison (treated vs. control) was done. In acute treatment, 5 control full sets of records were taken, one of the metals was injected ip., and the recording was continued for further ca. 2 hours. Recording and evaluation of the electrical activity was done by a PC, using the NEUROSYS software (Experimetria, UK). Individual records were averaged, and amplitude and latency of the main peaks measured (*Fig. 1A;* amplitude of the peaks A and B measured from the voltage zero line, latency from the stimulus artifact labeled with 0; see Papp et al., 2001).

The dynamics of the evoked responses were assessed on the basis of parameter changes during a series of stimuli. To this end, the first 5 and the last 5 potentials in a series were averaged. On these averages, amplitude and latency of the main positive and negative peaks in case of the somatosensory evoked potential, and peak latency only in case of the tail nerve potential, was measured and the ratio "last/first" calculated. These values were further group-averaged and the treated vs. control group were compared.

RESULTS

The most pronounced changes were seen on the amplitude of the cortical evoked response in the subacutely treated animals (*Fig. 1*). There was some amplitude decrease during the stimulus series also in the untreated control animals. In the Pb-treated group (*Fig. 1B*), the decrease of the amplitude of both peaks, and the peak-to-peak amplitude, was significantly stronger than in the controls ($p<0.05$, *t* test). The effect of Hg on the amplitude was minimal (*Fig. 1C*), that of Mn was more pronounced but was significant only on the peak-to-peak amplitude (*Fig. 1D*). Compared to the changes in the amplitude, the effect of the metals on the latency of the cortical evoked potential was minimal.

The effects of subacute heavy metal exposure on the evoked potential were compared to those of higher dose acute exposure. This was supposed to give an insight into how much time the subacute effect needs to develop which then indicates which of the known mechanisms of action may possibly be involved. Lead, again, had the strongest effect (*Fig. 2A*). The change, amplitude decrease, was similar to the effect of Pb in subacute exposure and developed to full size within 60 min. Hg also altered only the amplitude – a decrease, as in case of subacute exposure – but
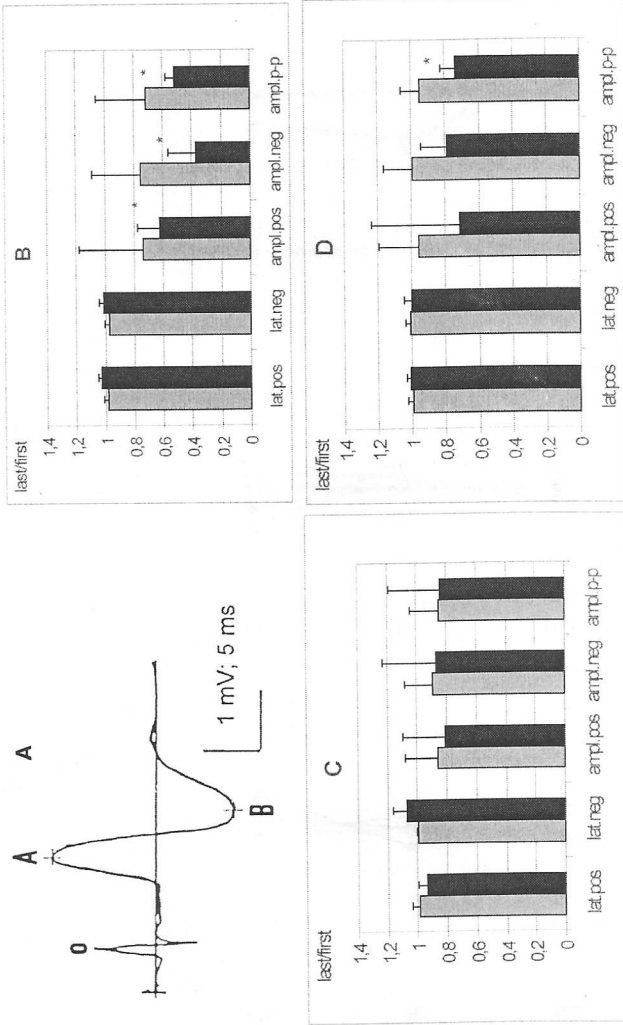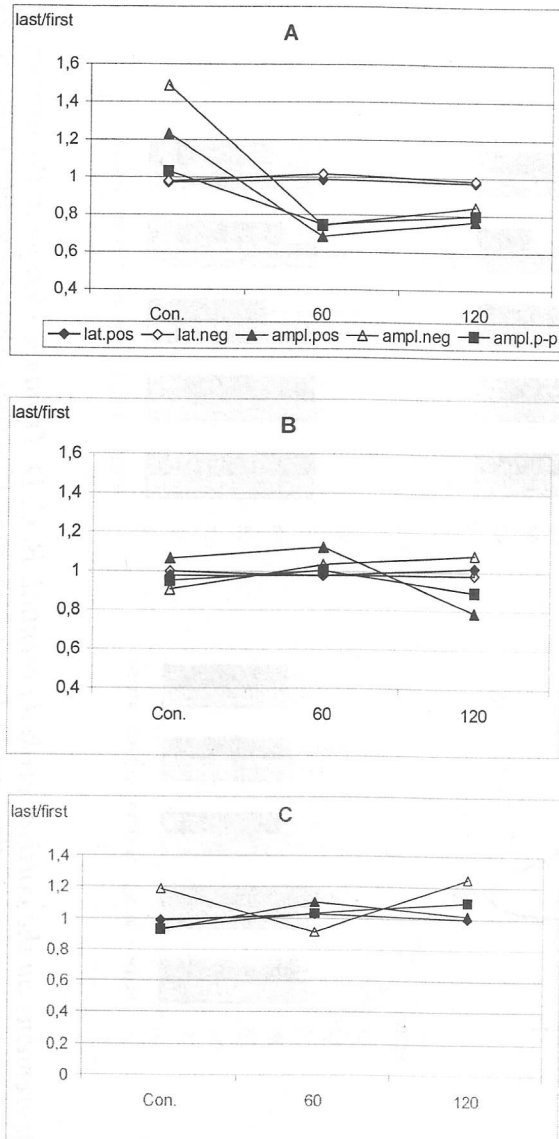
*Fig. 1. A: Measurements on the cortical evoked potential. B, C, D: Dynamic change in the latency and amplitude of the somatosensory cortical evoked potential after 10 weeks exposure to Pb2+ (B), Hg2+ (C), and Mn2+ (D). Ordinate: ratio of the average of the last 5/first 5 potentials in a series of 50. Light bars: untreated control, dark bars: metal treated (mean± S.D.). *: p<0.05 vs. control (t test).*

its effect was much weaker and less clear-cut (positive peak decreasing, negative peak increasing; *Fig. 2B*). The effect of Hg was virtually not yet present at 60 min after administration. The effect of Mn (*Fig. 2C*) was equivocal.

Fig. 2. Dynamic change in the latency and amplitude of the somatosensory cortical evoked potential after acute exposure to Pb^{2+} (A), Hg^{2+} (B) and Mn^{2+} (C). Displayed as in Fig. 1. *Con: pre-administration control, 60 and 120: 60 and 120 min after administration.*

An alteration of the cortical evoked response can, theoretically, arise anywhere between the peripheral receptor and the cortex. By comparing the heavy metal effects on a central (cortical evoked potential) and a peripheral (tail nerve action potential) form of activity, data concerning the localization of the effects could be obtained. To see a noticeable effect, stimulation frequency had to be set higher than for the cortical responses (up to 100 Hz) and it was found that the latency of the tail nerve action potential was relatively more affected than that of the cortical response (*Fig. 3A*). Here, Hg had the strongest, and Mn the weakest, effect. The response amplitude was decreased, most strongly by Pb and least strongly by Hg (*Fig. 3B*).
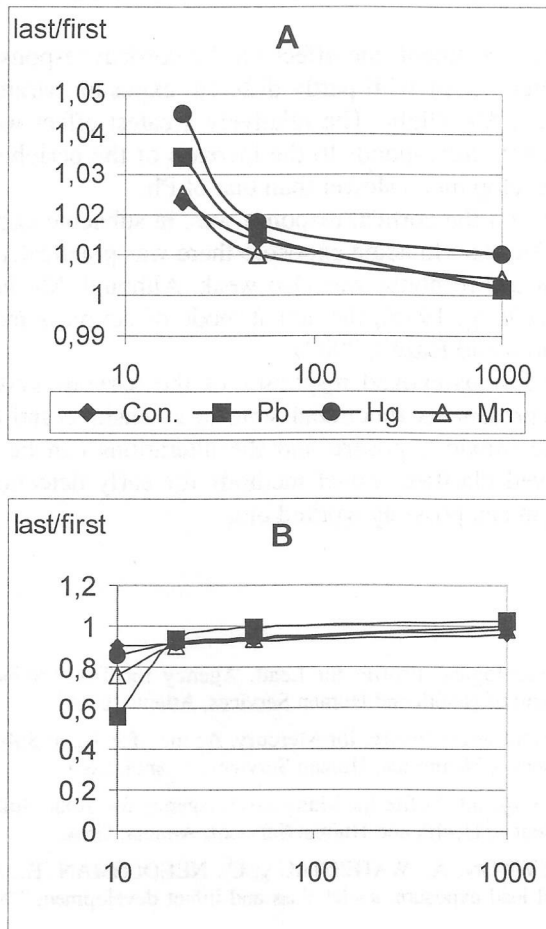

Fig. 3. Dynamic change in the latency (A) and amplitude (B) of the tail nerve action potential after a 10-week subacute exposure to Pb^{2+}, Hg^{2+}, and Mn^{2+}(insert), at 1000, 50, 20, and 10 ms stimulation period time (abscissa).

DISCUSSION

The effect of subacute exposure to the three metals, lead, mercury and manganese, on the amplitude and latency change during a stimulus series was of similar direction but different magnitude. The effect of Pb was the strongest and, based on comparison of the effect of acute and subacute exposure, did not need much time to develop. This means that the action of lead was not hindered by slow absorption (ATSDR, 1999a) and was possibly dependent on some ion channel effect (Büsselberg, 1995). The considerable reduction of the peripheral nerve action potential was in concordance to that but there was a contrast between the minimal effect on the cortical response latency and the manifest increase of the nerve action potential latency.

In case of the Hg treatment, the effect on the cortical response parameters was (in contrast to earlier results with partly different exposure parameters; Dési et al., 1996; Schulz et al., 1997) slight. The relatively greatest effect was on the latency, which, to some extent, corresponds to the increase of the peripheral response. The effect seemed to develop much slower than that of Pb.

The effect of Mn on the cortical response was, in subacute exposure, visible but mostly below significance. In acute exposure there was practically no effect and the effect on the peripheral response was also weak. Although Mn is known to act on ion channels (Büsselberg, 1995), the actual mode of action is more likely a metabolic one (Normandin and Hazell, 2002).

Alterations of stimulus-evoked responses of the nervous system, as described above, probably represent its functional state in a sensitive and direct way. If the relationship between toxic exposure and the alterations can be verified, and the mechanisms involved clarified, novel methods for early detection of damages of environmental origin can possibly worked out.

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