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Rapid communication

Acute and subchronic effects of lead on the central and peripheral nervous systems in rats in combination with alcohol

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Abstract

Humans are exposed, either simultaneously or sequentially, to various chemicals, including the neurotoxicants lead and ethanol. The aim of the present work was to investigate the changes in the spontaneous cortical activity (electrocorticogram; ECoG) and in the stimulus-dependent evoked potentials (EPs) recorded from rats pre-treated with alcohol and treated with lead acutely (intraperitoneally) or subchronically (by gavage). The measured parameters were spectral composition of the ECoG, amplitude and the latency of the stimulus-evoked cortical potential, as well as compound action potential amplitude, conduction velocity, and relative and absolute refractory period in a peripheral nerve. With subchronic lead and alcohol treatment, significant increase in the frequency of spontaneous activity and slight decrease in the EP amplitude were seen. In acute administration, EP amplitude increased and conduction velocity of the tail nerve decreased significantly. Our results showed that, in a combined exposure situation which is likely to happen also in humans, the known effects of neurotoxic heavy metals can be more severe.

Keywords: Lead; Alcohol; Neurotoxicity; Electrocorticogram; Evoked activity; Combined exposure; Subchronic exposure; Acute exposure

1. Introduction

Years of use of lead in paint and gasoline resulted in widespread environmental contamination and accumulation of the metal and, as a consequence, in repeated episodes of lead poisoning (ATSDR, 1999; Rogan, 1995). A crucial aspect of human exposure is neurotoxicity.

The adverse effect of chronic lead exposure on mental development of children has been described by several authors. Impaired IQ and different behavioral abnormalities were found in children with elevated body lead content (Bellinger et al., 1989; Needleman and Gatsonis, 1990). Otto et al. (1985) found characteristic electroencephalogram (EEG) and auditory evoked potential (EP) alterations in schoolchildren after several years of exposure to lead. In animal models, lead treatment induced EEG disorders and learning disability in young rats (Kumar and Desiraju, 1992). In our previous work, similar results were found (Nagymajtényi et al., 1997, 2000).

Due to chemical similarity of the two metal ions, absorption of Pb^{2+} and its distribution within the organism is interconnected with that of Ca^{2+} at several points. This, in turn, results in interference with a number of Ca-dependent regulatory processes (Sandhir and Gill, 1993).

It has been more and more realized in recent years that absorption and accumulation of heavy metals, e.g., lead, and the susceptibility of animals and humans to their toxicity are influenced by a suite of nutritional, physiological, and environmental factors (Maranelli et al., 1990). One such factor is ethanol, the effect of which on the toxicity of the heavy metals has not yet been established clearly. In case of a number of other toxicants, alcohol has been reported to enhance carcinogenicity, mutagenicity, or hepatotoxicity

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(Strubelt, 1984). Heavy use of alcohol in humans is associated with elevated blood lead concentration (Dally et al., 1989; Shaper et al., 1982), possibly because ethanol enhances the absorption of lead (Flora and Tandon, 1987).

The aim of the work presented here was to investigate the changes of spontaneous and stimulus-evoked cortical and stimulus-evoked peripheral nervous activity recorded from rats acutely treated with lead after alcohol preexposure (for 4 weeks in the drinking water) and from rats treated with alcohol and lead for 12 weeks subchronically. The measured parameters were spectral composition of spontaneous cortical activity (electrocorticogram; ECoG), amplitude and latency of cortical EPs, and action potential amplitude and conduction velocity in a peripheral nerve.

2. Materials and methods

2.1. Animals and treatment

The experiments were done on adult male Wistar rats, kept under standard conditions with food and water ad libitum. For subchronic lead exposure, 10-week-old rats (220–250 g body weight) were used: 60 animals in six groups of 10. Oral treatment with lead by gavage (5 days per week) and/or with alcohol through the drinking water was done as given in Table 1. The drinking water was tapwater, plain for groups marked W (including the control group) or with ethanol added for groups marked E. (Pb^{2+} concentration in the tapwater was 4 μg/L (≪ 50 μg/L, the legal limit), as reported by the local public health authority.) This treatment was continued for 12 weeks (final body weight: ca. 400 g). The resulting alcohol exposure of the rats—based on the standard rat water consumption of 10 mL/kg b.w. a day (Harkness and Wagner, 1989; Olfert et al., 1993), the alcohol concentration in the fluid (5 v/v%), and the ca. 1/3 fluid consumption of the rats in the E groups compared to the rats in the W groups—was approximately 2.5 g/kg body weight a day. In the literature, 1–5 g/kg b.w. daily ethanol doses are mentioned, mostly given once a day by gavage (Gupta and Gill, 2000; Tandon and Flora, 1989). Our dosage seems to fit in this range, and continuous exposure may be a more realistic model of heavy human alcohol use.

Table 1
Treatment groups in subchronic and acute lead exposure

Substance	Dose	Group label
Subchronic		
Water control	—	W
Ethanol	5 v/v% in the drinking water	E
Lead acetate[a]	80 mg/kg Pb^{2+} b.w. by gavage	Pb80W
	320 mg/kg Pb^{2+} b.w. by gavage	Pb320W
Lead acetate + ethanol	80 mg/kg Pb^{2+} b.w. by gavage + 5 v/v% in the drinking water	Pb80E
	320 mg/kg Pb^{2+} b.w. by gavage + 5 v/v% in the drinking water	Pb320E
Acute[b]		
Water control	—	W
Lead acetate	500 mg/kg Pb^{2+} i.p. during recording	Pb500
	1000 mg/kg Pb^{2+} i.p. during recording	Pb1000
Lead acetate + ethanol	500 mg/kg Pb^{2+} i.p. during recording, to rats pretreated with 5 v/v% ethanol for 4 weeks	Pb500E

[a]$Pb(CH_3COO)_2$ dissolved in distilled water to 1 mL/kg volume. Controls received pure distilled water by gavage.

[b]An ethanol-control group was not necessary as evidenced by the results of subchronic exposure.

Acute lead exposure was performed in untreated rats and in rats pretreated with alcohol in the above-mentioned manner for 4 weeks. Here, Pb^{2+} (1000 or 500 Pb^{2+} mg/kg, dissolved as given in Table 1) was administered i.p. during recording (see below) and the groups consisted of 8 rats each (32 rats altogether).

2.2. Recording

On the day following the last gavage (subchronic experiment) or at the end of the 4-week alcohol pretreatment (acute experiment), the rats were prepared for electrophysiological recording. In urethane (1000 mg/kg b.w.; Bowman and Rand, 1980) anesthesia, the head of the rats was clamped in a stereotaxic frame and the left hemisphere was exposed. Wounds were sprayed with 10% lidocaine and the exposed cortex was covered with warm paraffin oil. After ca. 1 h recovery, a silver recording electrode was placed on the somatosensory projection area of the whiskers (barrel field; Tracey and Waite, 1995). The corresponding peripheral site (contralateral whisker pad) was stimulated by electric pulses (ca. 4 V, 0.05 ms, 1 Hz) delivered via a pair of needle electrodes. In the tail, a pair of stimulating needle electrodes was placed at the base and another pair was placed 50 mm distally for recording (Miyoshi and Goto, 1973). Recording and evaluation of the electrical activity was PC based, using the Neurosys 1.11 software (Experimetria Ltd., UK). The pattern of recording consisted of a 5-min ECoG taken from the barrel field, followed by EPs recorded by applying one train of 20 stimuli. Finally, compound action potential of the tail nerve was also recorded (applying a train of 10 stimuli). From the subchronically treated animals, each form of activity was recorded once and the animal was sacrificed by an overdose of urethane. In animals with acute lead treatment, at least four pre-administration control records were taken at 20-min intervals; then lead was administered via a peritoneal cannula, and further records were taken for ca. 3 h. (In previous studies (Pecze et al., 2004) it was found that after this time the

effects did not change further and the animal's general state began to deteriorate slowly. That is why the effects will be evaluated below on the basis of the records 160 min after acute Pb^{2+} dosing.) A group remained untreated with lead during the whole recording and served as parallel control.

2.3. Evaluation and statistics

From the ECoGs, band activity (standard, delta to gamma; Kandel and Schwartz, 1985) was automatically determined and the so-called ECoG index (Dési, 1983) calculated (relation of the low and high frequencies in the recorded ECoG; [delta + theta]/[$beta_1$ + $beta_2$]).

The EP records were automatically averaged and the latency and amplitude of EP measured on the screen by cursors. On the tail nerve action potential, conduction velocity was measured after averaging, based on the distance between the stimulating and the recording sites and the response latency. The relative and absolute refractory periods of the nerve were determined by double stimulation (Anda et al., 1984), based on the latency increase of the second nerve action potential following the first one.

To visualize the effects, relative changes were calculated from the data. In the case of subchronic treatment (Figs. 1A, 2A, 3A, C, and D), group averages (mean ± SD) were calculated, and these were normalized to the mean of the control (W) group (that is, all mean and SD data were divided by the control mean value). Data of the acutely treated rats were first normalized individually, taking the average of the four pre-administration records as reference value for normalization (this was necessary to eliminate rat-by-rat variations in the baseline values of the electrophysiological parameters). Then, these normalized data were averaged group by group; the bars in Figs. 1B, 2B, and 3B thus represent the change of the given parameter during the treated period in the different groups.

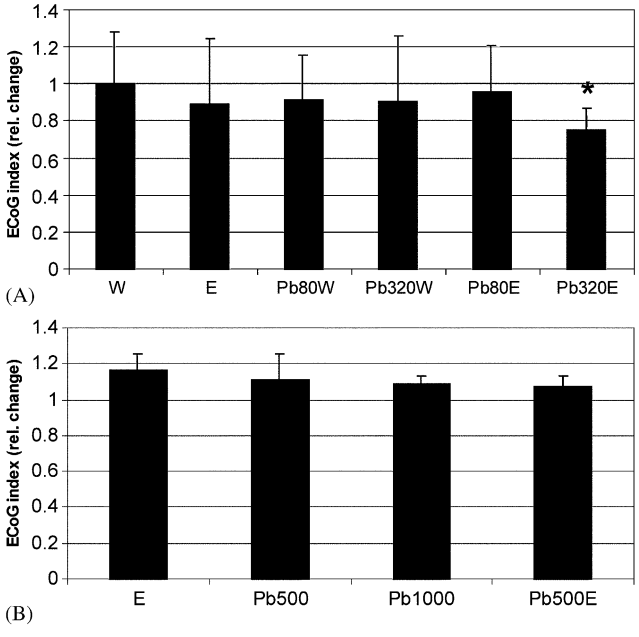

Fig. 1. Effect of subchronic (A) and acute (B) Pb^{2+} administration on the ECoG. Abscissa: dose groups as given in Table 1. Ordinate: change of the ECoG index (activity ratio of the slow/fast waves) in relative units (see Materials and methods for definition of ECoG index and for relative change calculation). The bars represent mean + SD. $^*P<0.05$ vs. control.

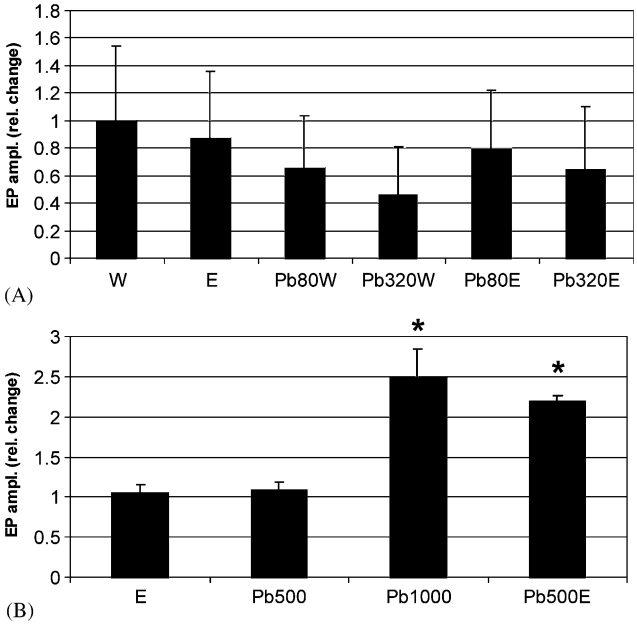

Fig. 2. Effect of subchronic (A) and acute (B) Pb^{2+} administration on the amplitude of the somatosensory cortical EP. Ordinate: amplitude in relative units. Displayed as in Fig. 1.

Statistical analysis was done by one-way ANOVA. Post hoc analysis of group differences was performed by subsequent LSD test, setting the significance level at $P<0.05$ in every case.

The whole study was performed in accordance with the principles of the Ethical Committee for the Protection of Animals in Research of the University.

3. Results

3.1. Effects on spontaneous cortical activity

In subchronic administration, alcohol, lead, and their combinations caused shifts to higher frequencies. In Fig. 1A, group averages of the ECoG index values (see Materials and methods) are given. The change was not significant, except for the ethanol + high dose lead (320 mg/kg) combination where the two toxicants seemed to be synergistic. In acute exposure, high dose lead (1000 mg/kg) caused a slight shift to lower

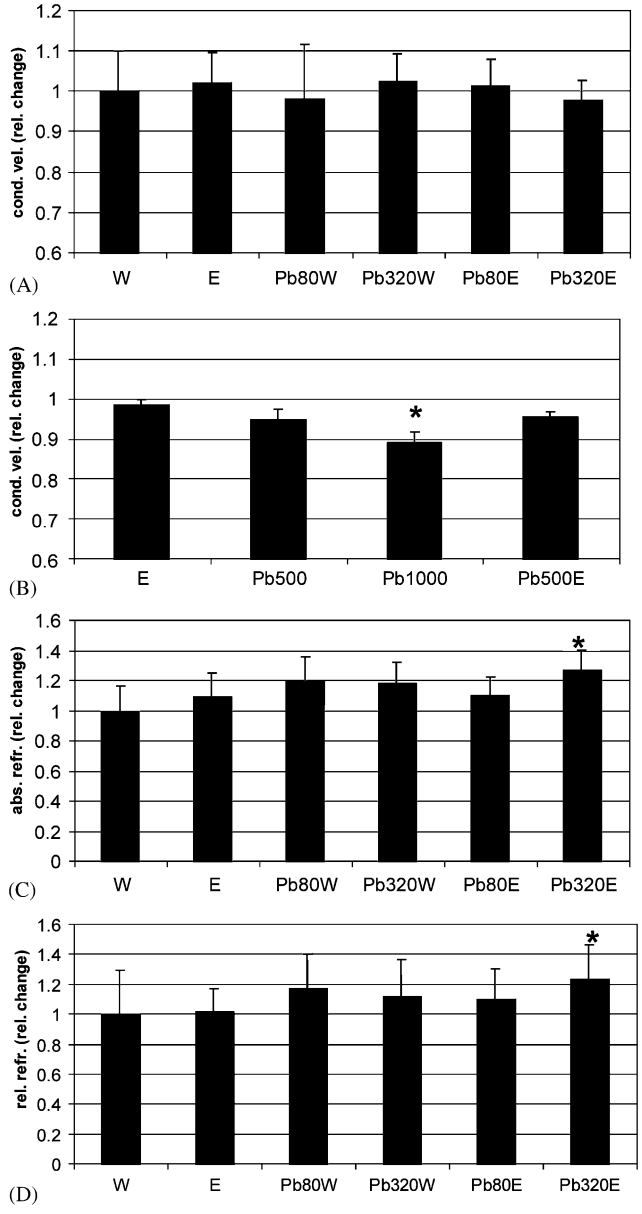

Fig. 3. Effect of subchronic and acute Pb^{2+} administration on the tail nerve compound action potential. (A and B) Conduction velocity in subchronic (A) and acute (B) Pb^{2+} administration (ordinate: relative units). (C) Absolute refractory period; (D) relative refractory period, in subchronic exposure (ordinate: relative units). Displayed as in Fig. 1.

frequencies in the spontaneous cortical activity (Fig. 1B). However, this effect was never significant and there seemed to be no interaction between Pb^{2+} and ethanol.

3.2. Effects on the somatosensory evoked cortical activity

In the evoked activity, latency and amplitude were the main parameters analyzed. The effect of lead and alcohol was primarily seen on the amplitude. In subchronic Pb^{2+} administration (Fig. 2A) there was a clear but nonsignificant decrease in the response amplitude. The effect was approximately dose dependent. In coexposure with alcohol, the amplitude decrease was, interestingly, less pronounced (in contrast to the effect on ECoG). On acute ip. dosing of high-dose Pb^{2+}, an increase in the response amplitude was seen (Fig. 2B) which evolved gradually over the recording period and was significant ($p<0.05$) vs. control from the 40th minute on. The acute low-dose Pb^{2+} (500 mg/kg) alone had no effect on the amplitude. In alcohol-pretreated rats, however, the increase of amplitude was similar to that obtained with the high-dose Pb^{2+} alone ($P<0.05$ vs. control).

The effect of Pb^{2+} on the latency of the cortical EP was below significance. In both subchronic and acute administration, only a slight increase was seen.

3.3. Effects on the peripheral nerve evoked activity

Lead in subchronic administration, alone or in combination with ethanol, had practically no effect on the conduction velocity of the tail nerve (Fig 3A). In acute treatment, however, a clear reduction of the conduction velocity of the tail nerve was seen (Fig. 3B), significant with the high-dose Pb^{2+} ($P<0.05$ vs. control). The low-dose Pb^{2+}, even in combination with ethanol, had a much weaker effect.

Two further parameters of the nerve conduction, relative and absolute refractory periods, were determined only in rats with subchronic lead exposure. Both parameters increased (became longer) in all treated groups but only the combination of high-dose Pb^{2+} and ethanol had a significant effect ($P<0.05$ vs. control; Figs. 3C and D).

4. Discussion

There was no qualitative difference between the effects obtained in the central and peripheral nervous system of rats by application of lead with and without ethanol. In the alcohol-pretreated rats, however, some of the effects were enhanced.

Lead, despite the hardly water-soluble salts formed by Pb^{2+} with physiological anions such as Cl^-, is readily absorbed by various routes of exposure (WHO, 1977). The blood–brain barrier cannot exclude Pb^{2+} above a concentration threshold (Bradbury and Deane, 1993) and will itself be damaged in the case of high blood lead level (Goldstein et al., 1974). Ethanol is known to increase the fluidity of cell membranes (Edelfors and Ravn-Jonsen, 1990) and to increase the permeability of the blood–brain barrier (Gulati et al., 1985). The result will be more lead entering the brain and affecting the neurons, as described in rats by Gupta and Gill (2000).

Several effects of the Pb^{2+} ion are explained by its chemical similarity to Ca^{2+}. Stimulus-evoked release of

ACh from rat brain synaptosomes was reduced (but spontaneous release increased) by Pb^{2+} (Suszkiw et al., 1984). By the same mechanism, Pb^{2+} can increase the ascending cholinergic cortical activation and shift the basal cortical activity to higher frequencies (Burdette and Goldstein, 1986; Kumar and Desiraju, 1992; Nagymajtényi et al., 1997). In subchronic administration, ethanol enhanced the effect of Pb^{2+} on the ECoG (in accordance with earlier results: Nagymajtényi et al., 2000), due possibly to higher Pb^{2+} level in the brain (see above) and to the effect of alcohol itself observed on human (Rangaswamy et al., 2002) and rat (Ehlers and Slawecki, 2000) EEGs. The lengthening of latency was probably due to decreased stimulus-dependent release of glutamate (Braga et al., 1999a, b) and decreased sensitivity of its cortical receptors (Ma et al., 1997). The GABAergic mechanism of alcohol, which caused the shift of spontaneous cortical activity to higher frequencies, could also disinhibit the cortical circuits involved in the generation of EPs (Koppell et al., 2000). This, in turn, would partially counteract the simultaneous depression (lower amplitude, lengthened latency) of the evoked cortical responses caused by Pb^{2+} (Nagymajtényi et al., 1997), which also explains why alcohol showed no synergism with Pb^{2+} on the evoked activity. Beyond that, Pb^{2+}, by acting on voltage-dependent Ca^{2+} and Ca^{2+}-activated K^{+}-channels (Audesirk and Audesirk, 1991; Leinders and Vijenberg, 1992; Reuveney and Narahashi, 1991), could slow the propagation of action potential, resulting in the observed effects on the peripheral nerve and contributing to the increased latency of the cortical response.

The effect of acute high-dose i.p. lead on the cortical EP was opposite to the effect seen in subchronic exposure. The increase of the amplitude was probably due to the lead-induced reduction of GABAergic inhibition, known to exist in vitro (Waskiewicz, 1996) and in vivo (Krishnamoorthy et al., 1993). The synergism with alcohol, observed when lead was given acutely in higher doses (Table 1), probably resulted from the already impaired GABAergic inhibition in the alcohol-pretreated rats (Koppell et al., 2000). Why this effect of Pb^{2+} acting to increase the amplitude of the EP was prevailing in the acute application and those acting to decrease it were prevailing in the subchronic application, remained unclear. One possible explanation would be differences in their dependence on local Pb^{2+} concentration.

Although the kind and mechanism of interaction between the neurotoxic lead and the alcohol, given to rats in our experiments, are not fully clear, our results showed that in a combined exposure situation, which is likely to happen also in humans, the known effects of neurotoxic heavy metals can be more severe.

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