

A study on electrophysiological effects of subchronic cadmium treatment in rats

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Abstract

Male Wistar rats were treated for 4, 8 and 12 weeks with 3.5, 7.0 or 14.0 mg/kg cadmium (in the form of cadmium chloride) by gavage. Changes induced in certain electrophysiological parameters—electrocorticogram frequency; latency and duration of cortical sensory evoked potentials; conduction velocity and relative and absolute refractory periods of a peripheral nerve—were analyzed. On the electrocorticogram, increased frequency was seen. Lengthened latency and duration of the cortical evoked potentials, as well as lowered conduction velocity and increased refractory periods in the peripheral nerve, were observed. These changes seemed to increase with the dose and the treatment time and were statistically significant mainly in the highest dose groups following 12 weeks of treatment. The results show that subchronic, low-level exposure by cadmium affects the rat's spontaneous and evoked bioelectric activity and point at the possible consequences in exposed humans.

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1. Introduction

Cadmium is one of the heavy metals with a number of toxicological implications. In modern technology, there are numerous applications of cadmium and its derivatives resulting in risk of occupational and/or environmental exposure (Elinder, 1992). Occupational exposure is found in industries smelting and processing the metal, in battery manufacturing, etc. (ATSDR, 1999). The population is mainly exposed via ingestion of the Cd content present in food, such as cereals and bread, as well as meat, fish, eggs, oils, rice, fruits and vegetables and in drinking water (Muller and Anke, 1994; Smigiel, 1994). Near factories with high Cd emission, house dust and home-grown vegetables can also be an important source of human exposure (Alonso et al., 2001; Leroyer et al., 2001). Smokers have, in addition, a chronic Cd load originating from the Cd contained in the tobacco

which is easily absorbed (to $\approx 50\%$) from the lungs (Elinder et al., 1983).

Toxic effects of Cd were reported by several authors both from animal experiments and human epidemiological studies. The most important target organs in Cd intoxication are the kidneys, the liver, the bones and the respiratory and cardiovascular system (WHO, 1992; ATSDR, 1999). Beside these effects, Cd exposure can also cause behavioral and neurological disorders. In occupational settings, reduced visuomotor performance and difficulties of concentration and postural balance were observed (Viaene et al., 2000). In children exposed to Cd, effects on higher nervous functions, such as lowered IQ (Thatcher et al., 1982; Jiang et al., 1990) or behavioural abnormalities (Marlowe, 1986), were found. In rats exposed to Cd before and/or after birth, locomotor (Smith et al., 1985; Ruppert et al. 1985) and behavioural (Mohd et al., 1986) development was affected. Repeated doses of Cd to rats for 9 days caused epileptiform EEG activity (Vataev et al., 1994). Cd modifies the function of Ca channels and is used as a Ca channel blocker in in vitro experiments (Calabresi et al., 1990; Soliakov and Wonnacott, 1996). Effects on transmitter metabolism (acetylcholine: Devi and Finger-

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man, 1995; biogenic amines: Flora and Tandon, 1987) have also been described.

The aim of this study was to investigate the neurophysiological effects of a subchronic, low-level cadmium exposure in rats and to compare the sensitivity of the investigated neurotoxicological parameters to Cd, with the final goal of finding sensitive biomarkers for early alterations of the brain.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats (10-week-old) were used for our experiments. The animals were kept under conventional conditions (temperature 20–22 °C, humidity 60–70%, 12 h light:dark cycle) and fed with standard rodent chow. Food and water were available at all times.

The animals (ten per dose group) were treated through gavage by 3.5, 7.0 or 14.0 mg/kg b.w. of cadmium (CdCl₂ of 99.5% purity, dissolved in distilled water and administered in 1 ml/kg b.w. volume) for 4, 8 or 12 weeks, in a 5 days per week system. Control rats received the same volume of distilled water. The animals were observed daily for symptoms of intoxication and body weight was weekly recorded.

2.2. Neurophysiological investigation

The electrocorticogram (ECoG) and the sensory evoked potentials were recorded 1–2 days after the last Cd administration. The rats were anaesthetized with urethane (1000 mg/kg i.p.; Bowmann and Rand, 1980) and placed in a stereotaxic frame. The skull was opened and silver electrodes were placed on the primary somatosensory, visual and auditory cortical foci (Par1, OclB, Tel areas, respectively; Zilles, 1982). Later (30 min), the ECoG was simultaneously recorded from these areas for 5 min. The records were stored on PC, band activity (delta–gamma; Kandel and Schwartz, 1985) was determined by the software and 'EcoG index' (slow/fast band activity ratio: $(\delta + \theta)/(\beta_1 + \beta_2)$; Dési, 1983) was calculated.

Then, cortical evoked potentials were recorded through the same electrodes. Somatosensory stimulation was carried out by means of an electrode pair pricked into the whiskery part of the skin through which rectangular electrical stimuli (1 Hz, 3–4 V, 0.2 ms) were delivered. The visual stimuli were flashes (1 Hz, 60 lux) lead from a flash bulb unit via an optical fiber directly to the contralateral eye. Acoustic stimulation was performed by clicks (1 Hz, 40 dB), produced by a small earphone put into the ear of the rats. Of each modality, 50 evoked potentials were stored and averaged. Latency and duration of the averaged evoked

Table 1
Body weight, brain weight and relative organ weights (weight of an organ divided by that of the brain) in the groups treated with the doses and for the periods indicated

Treatment time	Dose (mg/kg)	Body weight (g)	Brain weight (g)	Relative organ weights						
				Liver	Lung	Heart	Kidney	Spleen	Thymus	Adrenals
4 weeks	Control	286±19	1.66±0.10	7.51±0.41	1.06±0.04	0.60±0.03	1.33±0.05	0.34±0.04	0.24±0.03	0.03±0.002
	3.5	280±13	1.64±0.10	7.48±0.34	1.04±0.03	0.60±0.02	1.32±0.05	0.33±0.02	0.23±0.02	0.02±0.002
	7.0	292±25	1.58±0.09	7.45±0.33	1.02±0.02	0.61±0.03	1.34±0.06	0.33±0.03	0.21±0.03	0.03±0.002
	14.0	288±24	1.59±0.08	7.42±0.27	1.04±0.03	0.63±0.02	1.34±0.03	0.31±0.03	0.22±0.03	0.02±0.002
8 weeks	Control	351±18	1.74±0.12	7.50±0.36	1.05±0.04	0.62±0.04	1.35±0.07	0.31±0.04	0.24±0.03	0.03±0.002
	3.5	354±28	1.79±0.11	7.45±0.32	1.03±0.04	0.63±0.02	1.34±0.05	0.32±0.02	0.21±0.03	0.03±0.002
	7.0	325±13	1.72±0.13	7.43±0.35	1.04±0.02	0.61±0.02	1.40±0.04	0.32±0.03	0.20±0.04	0.03±0.002
	14.0	337±16	1.69±0.10	7.41±0.26	1.04±0.02	0.61±0.03	1.40±0.04	0.33±0.04	0.22±0.03	0.03±0.002
12 weeks	Control	429±48	1.84±0.10	7.54±0.33	1.06±0.03	0.61±0.03	1.33±0.05	0.33±0.03	0.22±0.03	0.03±0.002
	3.5	417±53	1.82±0.11	7.43±0.34	1.04±0.03	0.60±0.04	1.40±0.06	0.34±0.02	0.21±0.03	0.03±0.003
	7.0	410±42	1.83±0.07	7.41±0.43	1.03±0.02	0.60±0.03	1.42±0.04	0.31±0.022±0.04	0.03±0.002	
	14.0	382±41	1.89±0.11	7.38*±0.31	1.02±0.05	0.61±0.04	1.43±0.04	0.34±0.02	0.21±0.02	0.03±0.002

Mean±S.D.

* $P < 0.05$, compared to the corresponding control.

potentials was measured afterwards, using the program's measuring cursors on the monitor screen.

The conduction velocity of the tail nerve was measured by the modified Miyoshi method—instead of 37 °C, recording was made at 21–22 °C (Miyoshi and Goto, 1973). The relative and absolute refractory periods were calculated according to Anda et al. (1984). Urethane anesthesia was maintained over the whole recording procedure for both ethical (see below) and technical reasons. Although all anesthetics are known to affect cortical electrical phenomena (Angel and Gratton, 1982; Hayton et al., 1999), the influence of urethane on the results was most probably minor because all animals, treated or control, underwent the same preparation.

After all electrophysiological recordings, the rats were sacrificed by an overdose of urethane and the weight of brain, liver, heart, lung, kidneys, thymus and adrenal glands was measured. Relative organ weights, related to the brain weight, were calculated.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

2.3. Statistics

After checking for normality by means of the Kolmogorov–Smirnov test, the data were analyzed by univariate ANOVA. Post hoc analysis of group differences was performed by a subsequent LSD test setting the probability level at $P < 0.05$. Possible trends in the Cd-induced alterations were tested by calculating correlation coefficients.

3. Results

3.1. General effects

There was a slight decreasing tendency in the treated animals in their body weight gain and the relative organ weights, but this was never significant (Table 1). The only exception is the relative liver weight showing a significant dose-dependent decrease after 12 weeks of treatment with the highest dose ($P = 0.0465$). These data, together with the observation of the animals alive and the organs after dissection, indicated no overt Cd intoxication in any of the treated groups.

3.2. Neurophysiological effects

Compared to the controls, the effect of Cd treatment on the spontaneous cortical activity was similar in the three centers (no significant difference between any two centers was seen within the same treatment dose and time group), with the somatosensory area showing the

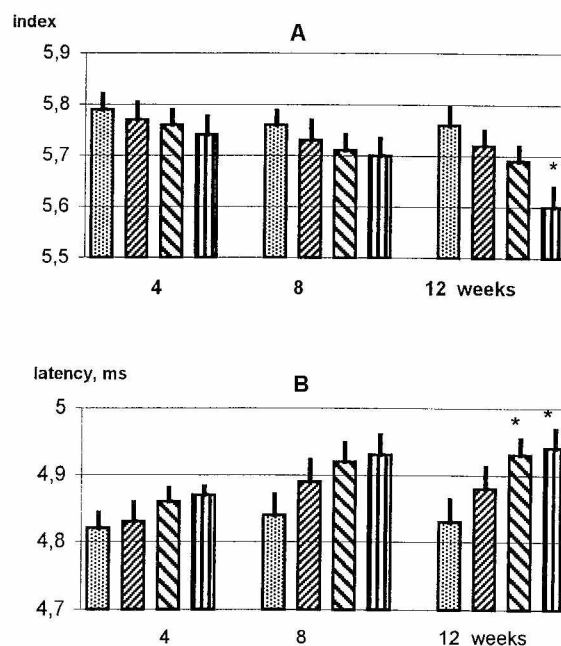


Fig. 1. Changes of the ECoG index (A) and evoked potential latency (B) on subchronic cadmium treatment in the somatosensory cortical area. Ordinate: index value (A) and latency in ms (B). Abscissa: treatment time in weeks. Error bar: S.D. Groups indicated by the bar filling: dotted, control; fine hatched, 3.5 mg/kg; coarse hatched, 7 mg/kg; striped, 14 mg/kg. * $P < 0.05$ compared to the corresponding control.

most marked alterations. Decreased amplitude and increased mean frequency was seen, with a tendency of dose- and time-dependence, but below significance ($P > 0.05$). ECoG index, however, proved to be more sensitive: treatment with the highest Cd dose for 12 weeks caused a significant decrease of the index in the somatosensory ($P = 0.0211$; Fig. 1A) as well as in the visual ($P = 0.0222$) and the auditory ($P = 0.0117$) center. With lower dose and/or treatment time the change of the index was similar but not significant (Table 2, upper panel).

The latency and duration of all evoked potentials were lengthened in a dose- and time-dependent manner. Latency of main waves of the somatosensory evoked potentials increased at all doses and treatment times, but these differences were significant only with the high ($P = 0.0101$) and the medium dose ($P = 0.0479$) at the end of the 12 weeks period (Fig. 1B). Longer interpeak duration of the waves, but without statistical significance, were also observed. In the visual evoked potentials, latency of wave N2 became more expressed with the time in all the treated groups and was significant in the group receiving the highest dose for 12 weeks ($P = 0.0265$). Longer duration between the peaks was also seen, but this was never significant. Similarly, the latencies of the auditory evoked potential waves were longer than the control for all treatment times; in the case of wave N1, the alteration was significant at the

Table 2

ECoG index values and latency of the sensory evoked potentials recorded from the three cortical foci

Treatment time	Dose (mg/kg)	Cortical area		
		Somatosensory	Visual	Auditory
<i>ECoG index</i>				
4 weeks	Control	5.79±0.03	4.18±0.04	4.23±0.036
	3.5	5.77±0.033	4.17±0.045	4.22±0.04
	7.0	5.76±0.028	4.16±0.036	4.17±0.05
	14.0	5.74±0.035	4.12±0.044	4.10±0.066
8 weeks	Control	5.76±0.026	4.17±0.05	4.24±0.04
	3.5	5.73±0.038	4.14±0.043	4.20±0.047
	7.0	5.71±0.029	4.12±0.038	4.16±0.068
	14.0	5.7±0.032	4.09±0.065	4.08±0.07
12 weeks	Control	5.76±0.033	4.17±0.044	4.25±0.03
	3.5	5.72±0.029	4.10±0.035	4.19±0.03
	7.0	5.69±0.028	4.04±0.055	4.15±0.063
	14.0	5.60±0.037*	4.03±0.040*	4.03±0.060*
<i>Sensory evoked potential latency (ms)</i>				
4 weeks	Control	4.82±0.02	107.5±3.51	54.6±0.83
	3.5	4.83±0.03	108.0±4.20	55.0±0.90
	7.0	4.86±0.02	108.5±2.68	55.6±1.05
	14.0	4.87±0.01	109.5±3.5	55.9±0.77
8 weeks	Control	4.84±0.03	107.0±3.30	54.3±0.64
	3.5	4.89±0.03	108.5±4.01	55.4±0.77
	7.0	4.92±0.02	109.0±2.70	55.8±0.94
	14.0	4.93±0.04	110.5±3.27	56.2±0.70
12 weeks	Control	4.83±0.03	107.5±2.90	54.5±0.86
	3.5	4.88±0.03	109.0±3.92	55.9±0.65
	7.0	4.93±0.02*	109.5±2.60	56.3±0.60
	14.0	4.94±0.03*	111.5±3.03*	57.0±0.75*

Mean±S.D.

* $P < 0.05$ compared to the corresponding control.

highest dose and 12 weeks of treatment ($P = 0.0248$). See Table 2 (lower panel) for latency changes in the three cortical foci.

The conduction velocity of the peripheral nerve was significantly decreased in the groups treated with the two higher doses for 12 weeks (high dose: $P = 0.0204$; medium dose: $P = 0.0421$; Fig. 2A). In the same groups, the relative and absolute refractory periods were also significantly longer (relative, high dose: $P = 0.0010$, medium dose: $P = 0.0197$; absolute, high: $P = 0.0027$, medium: $P = 0.020$; Fig. 2B,C).

4. Discussion

In our experiment, the effect on the body weight gain and the relative organ weights of up to 14 mg/kg per day Cd given by gavage was mostly slight, except for the relative liver weight. With a dosing regime comparable to ours, Brzoska et al. (2002) found a significant decrease of the liver weight, as we did, and a liver Cd level of $\approx 15 \mu\text{g/g}$. Shibutani et al. (2001), with 40 $\mu\text{g/g}$

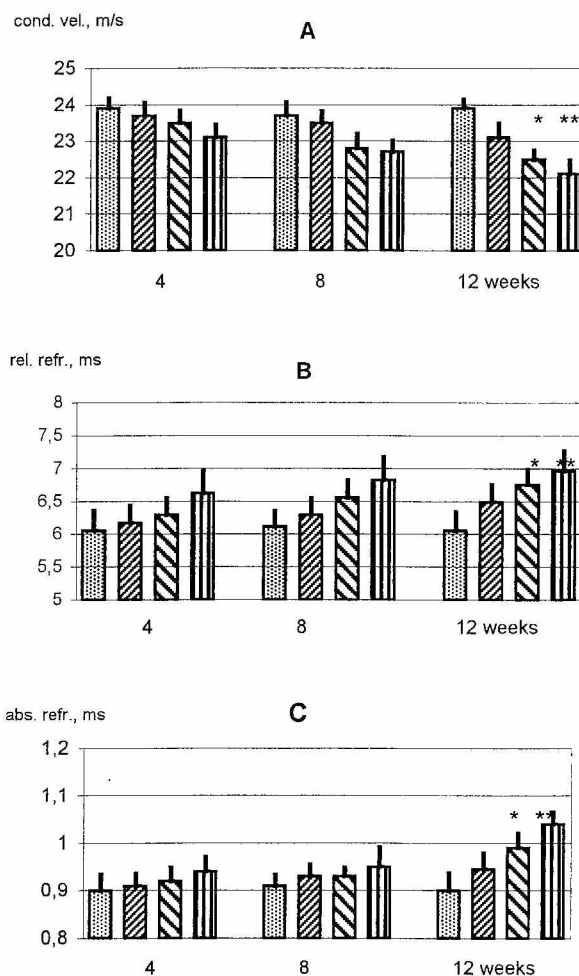


Fig. 2. Changes in the functional parameters of the tail nerve induced by subchronic cadmium treatment (A: conduction velocity; B: relative refractory period; C: absolute refractory period). Ordinate: conduction velocity in m/s (A), relative refractory period in ms (B) and absolute refractory period in ms (C). The same display as in Fig. 1. * $P < 0.05$, ** $P < 0.01$ compared to the corresponding control.

CdCl_2 in the food given to rats for 18 months, obtained $\approx 130 \mu\text{g/g}$ liver Cd level. These liver levels are in the range of those described from workers with long-term occupational exposure to Cd (Roels et al., 1981) and suffering from peripheral neuropathy (Viaene et al., 1999). This is in line with our findings of neurophysiological alterations in absence of major general toxic effects of Cd.

Cd-induced alterations of the spontaneous cortical activity have been published in the literature. Vataev et al. (1994) reported significant changes of EEG recorded from the somatosensory, visual and auditory cortical areas and from the hippocampus after single or repeated administration of Cd to rats in doses comparable (or lower) than those applied by us.

In spite of its low permeability across the blood–brain barrier, Cd was described to accumulate in various parts of the rat brain after 2 months oral exposure by $\approx 5 \text{ mg/kg}$ body weight, a dose comparable to ours (Clark et al.,

Table 3

Correlation coefficients (R^2) indicating the trends of dependence of the investigated electrophysiological parameters (dependent variables) on time and dose (independent variables)

Dependent variable	Independent variable	Dose (at 12 weeks)	Time (high dose group)	Time (control group)
EcoG index	Somatosensory	0.9956	0.9425	0.7100
	Visual	0.8171	0.9643	0.7231
	Auditory	0.9902	0.9231	0.7500
Evoked potential latency	Somatosensory	0.8197	0.8547	0.2500
	Visual	0.9791	0.9956	0.5768
	Auditory	0.8673	0.8547	0.1021
	Conduction velocity	0.8969	0.9868	0.0500
Peripheral nerve	Relative refractory period	0.8738	0.9867	0.0805
	Absolute refractory period	0.9746	0.8242	0.2385

Dose dependence was tested after 12 weeks treatment. Time dependence was tested across the period of 0–12 weeks in the control and the high dose groups.

1985). In our experiments, the alteration of the spontaneous activity recorded from different cortical areas seemed to become more expressed with increasing dose and treatment time (Table 3), i.e. with the total amount of cadmium ingested. This suggested a correlation between the extent of the electrophysiological effect and the amount of Cd accumulated in the brain. On the other hand, Cd probably affected all parts of the investigated sensory pathways as witnessed by the qualitatively and quantitatively similar alterations in the parameters of the sensory evoked potentials seen in our experiments (Table 2). Sensory systems seem generally to show a specific sensitivity to Cd, evidenced by the high accumulation of the metal in the thalamus and olfactory bulb in treated rats (Clark et al., 1985) or the reduced brainstem auditory response (Whitworth et al., 1999).

At the level of neurons, several aspects of the toxic effects of Cd have been described. Cd has been shown to influence the metabolism of monoamines (Hobson et al., 1986; Flora and Tandon, 1987) and acetylcholine (Devi and Fingerman, 1995). It was observed in rat amygdala that excitatory transmitters were more affected by Cd administration than inhibitory ones (Minami et al., 2001). Through its well-known blocking effect on calcium channels, Cd can affect transmitter release (Soliakov and Wonnacott, 1996) or other calcium regulated phenomena. The effect on monoamines and acetylcholine, as modulators of cortical activity, can explain the alterations found in the ECoG.

Another possible explanation of the effects seen on the cortical evoked potentials is the ion channel effect of Cd. It is known that Cd blocks certain ionic channels (Nelson, 1986). This way, the conduction of the action potential can be slowed both in the peripheral nerves (see Viaene et al., 1999, 2000) and the central paths. As a consequence, the latency and also the interpeak duration of evoked potentials will be lengthened.

Human epidemiological studies have shown that large populations are continuously exposed to Cd, either occupationally or environmentally, by drinking water and food and especially by smoking (ATSDR, 1999). The affected persons can have, consequently, a higher risk of behavioral and functional neurotoxic disorders. On the basis of previously reported data and of our results, we suppose that application of sensitive and non-invasive screening methods (such as EEG and/or cortical evoked potential recording) would be useful to detect early signs of Cd intoxication at population level. By combining Cd level measurements with recording of those electrophysiological parameters which proved to be the most sensitive (such as the parameters of the peripheral nerve conduction in our experimental system) appropriate biomarkers of the low dose human exposure could be developed.

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