CHANGES IN CERTAIN DYNAMIC FEATURES OF SENSORY EVOKED POTENTIALS OF RATS ON EXPOSURE TO METAL XENOBIOTICS

ANDRÁS PAPP[1], TERKEN BAYDAR[2], TÜNDE VEZÉR[1], AND LÁSZLÓ NAGYMAJTÉNYI[1]

[1] Department of Public Health, Faculty of General Medicine, University of Szeged Medical and Pharmaceutical Center, Hungary

[2] Department of Pharmaceutical Toxicology, Hacettepe University, Ankara, Turkey

ABSTRACT: In modern societies, whole populations are exposed to a variety of toxic substances, several of which affect the nervous system. This points to the need of searching for means of early detection of harmful effects. Sensory evoked potentials are readily recorded in experimental animals and in humans and have been shown to be sensitive of toxic effects. Taking rats subchronically exposed to different environmental xenobiotics as a model of human exposure, our aim was to identify toxicant-induced changes, which can be used for early detection of neurotoxic effects in humans. Young adult rats were treated for 10–12 weeks with various insecticide agents and heavy metals, and sensory evoked potentials were recorded after the treatment period in acute experiment. Dependence of the basic parameters of the evoked potentials, i.e. amplitude, latency and duration, on the frequency of stimulation and on the number of stimuli in a train was investigated.

In the somatosensory evoked potentials there was a slight frequency dependence on the latency and duration, which was dissimilar in control and treated rats. The sequence of stimuli (first/last ones) had an effect mainly on the amplitude of somatosensory and visual evoked potentials, which again was altered by the xenobiotic treatment. It can be concluded that certain properties of the evoked potentials seem to be highly sensitive to xenobiotic exposure and may be usable for detection of such exposures in humans.

KEY WORDS: Organophosphates, heavy metals, neutotoxicity, evoked potentials, biomarker, rat

INTRODUCTION

Xenobiotics entering the human body from the environment and via food and drink are a major source of health risk. As heavy metals – including lead, mercury and cadmium – continue to be used in industry, agriculture and medicine, ill effects due to them deserve special attention. Several environmental heavy metal compounds are known to affect the nervous system, our knowledge on their possible population neurotoxic effects at low doses and long exposure times is, however, limited.

Corresponding author: András Papp
SZTE Department of Public Health
Dóm tér 10.
H-6720 Szeged, Hungary
Fax: (+36) 62 545 120
E-mail: ppp@puhe.szote.u-szeged.hu

The adverse effect of chronic inorganic lead exposure (WHO, 1995) has been described by several authors. Needleman (1983), Needleman and Gatsonis (1990) and Bellinger et al. (1989) found impaired IQ and various behavioral difficulties in children with elevated body lead content due to chronic exposure. Otto et al. (1985) observed characteristic EEG and auditory evoked potential alterations in school-children after several years of exposition to lead. From a large-scale multicenter study, similar alterations were reported by Winneke et al. (1990). In animal models, postnatal lead treatment induced EEG disorders and learning disability in young rats (Kumar and Desiraju, 1992). Lead-exposed rhesus monkeys had diminished discriminative auditory performance (Molfese et al., 1986). Alterations of sensory evoked potentials of rats on subchronic lead exposure was reported by Nagymajté-nyi et al. (1997b).

Mercury (WHO, 1991) as well is known to affect human and animal nervous system whereby the effects of organic and inorganic mercurials were in some cases found to be similar (Yuan and Atchison, 1994). Mercuric chloride was found to cause impaired learning together with behavioral abnormalities in young rats (Archer, 1993). Human occupational exposure to Hg vapor, resulting in various abnormalities of the peripheral (Singer et al., 1987) and central (Lille et al., 1988; Soleo et al., 1990) nervous system functions, has been extensively described. In monkeys, methyl mercury caused sensory deficits (Rice, 1996). Alteration of spontaneous and evoked cortical activities on exposure to inorganic mercury in rats was described by Schulz et al. (1997).

Cadmium (WHO, 1992) is known to affect several organs including the liver, the kidneys and the skeletal system. Its neurotoxic effect, however, has not been described until rather recently. It turned out that pre- and/or postnatal Cd exposure affects locomotor (Ruppert et al., 1985; Smith et al., 1985) and behavioral (Mohd et al., 1986) development in young rats and alters some functional features of spontaneous and evoked CNS activities (Yargicoglu et al., 1996; Nagymajtényi et al., 1997a). Cadmium also influences the metabolism of acetylcholine (Devi and Fingermann, 1995) and of biogenic amines (Flora and Tandon, 1987) in the brain and has possibly a role in nervous tissue degeneration due to abnormal tissue oxidation (Shukla et al., 1987; Kumar et al., 1996) and mitochondrial respiration (Fern et al., 1996).

Aluminum has been suspected to be a causal or confounding factor in human neurodegenerative processes like Alzheimer's disease (WHO, 1997). Coordination and manipulation was impaired in exposed workers (Hosovski et al., 1990). The well-known "dialysis encephalopathy" is also attributed to the dialysate-borne aluminum exposure (Schreeder at al., 1983). Impairment of learning and memory was found also in aluminum-treated rabbits (Solomon et al., 1990) with possibly corresponding electrophysiological changes (Franceschetti et al., 1990).

Given the alterations of evoked central nervous activity on exposure to most of the metals mentioned above, both in humans and in experimental animals, it seemed worthwhile to test whether such alterations can be used for early recognition of toxic exposure, i.e. as a biomarker (Grandjean et al., 1994). To this end, certain "dynamic features" of the cortical evoked potentials were defined. Changes of the

parameters of the individual evoked potentials (increase of latency, decrease of amplitude) over a series of 50 stimuli were considered as "fatigue". "Frequency dependence", on the other hand, was defined as dependence of the evoked potential parameters on the frequency of stimulation. It was investigated whether these features are significantly different in metal exposed and control rats.

METHODS

The investigation was based on the method of recording described previously (Nagymajtényi et al., 1997a,b). Rats (Wistar males, 10 weeks old) were treated by gavage, 5 times per week for 12 weeks, with 320 mg/kg b.w. lead (lead acetate), 1.6 mg/kg mercury (mercuric chloride), 14 mg/kg cadmium (cadmium chloride) or for 6 weeks with 200 mg/kg aluminum (aluminum chloride). Controls had distilled water in the gavage. At the end of the treatment period, the animals were anesthetized with 1000 mg/kg urethane, the head was fixed, and the left hemisphere exposed. Somatosensory and visual cortical evoked potentials were recorded from the corresponding primary foci. The stimulus was an electric square pulse (ca. 3 V, 0.05 ms) delivered to the right whisker pad or a 60 lux flash directed to the contralateral eye by a glass fiber light conductor. Stimulation was performed in series of 50 pulses at various frequencies.
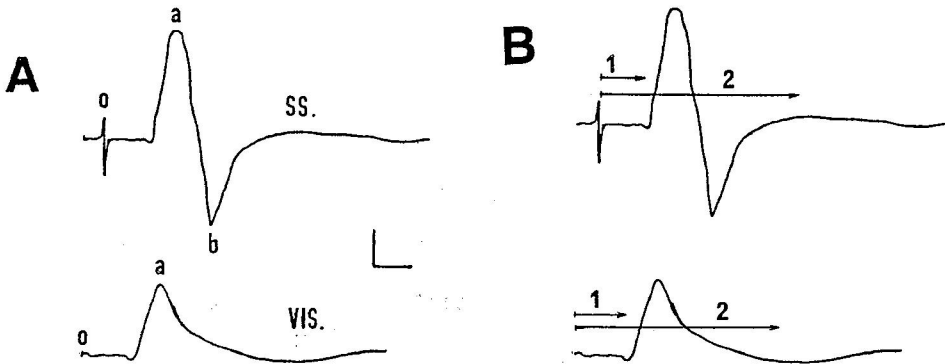

Fig. 1. Measurements on the averaged sensory (SS: somatosensory, VIS: visual) evoked potentials.
A: Peak amplitude and latency was measured, in vertical and horizontal direction, respectively, between point o (time zero and voltage zero level) and points a and b (SS) or point a (VIS). For SS, zero was marked by the stimulus artifact, for VIS, it was taken from the trigger zero.
B: Onset latency of both evoked potentials was measured as shown by the length of arrow 1. Arrow 2 extends to the cessation point of the principal wave(s). Duration is obtained by subtracting. Calibration: 0.5 mV, 5 ms for SS; 0.5 mV, 50 ms for VIS.

For the evaluation of "fatigue", a stimulation frequency of 1 Hz was used. The first 5 and the last 5 of the evoked potentials of a series (stored in a PC) were averaged, and amplitude and latency of the main negative and positive wave component peaks was measured (Fig. 1A). The relative change ([average of the last 5] / [average of the first 5]) indicating contingent fatigue was calculated and again averaged for the whole groups of animals (10 rats). For determination of frequency dependence, series of 50 stimuli were applied at 1, 2 and 5 Hz frequency, and the onset latency and the duration of the evoked potentials were measured (Fig. 1B) and again, the average values for the groups were calculated. Presence and significance of the difference in fatigue or frequency dependence between the control and treated groups was tested with two-sample t-test (average was calculated).

RESULTS

Of the parameters investigated the latency of the main waves of the evoked potentials showed minor changes during a series of stimuli. Of the metals tested, only lead caused a stronger increase of the latency of the negative peak of the somatosensory evoked potential. The amplitude changes over the series were, to the contrary, clear-cut, and hence interpretable as fatigue.

TABLE 1. Group averages of the fatigue, calculated as relative change, in various parameters of the evoked potentials. Values above and below zero indicate that the given parameter was increased and decreased, respectively, in the last potentials of a series vs. the first ones. * p<0.05

Somatosensory evoked potential

Relative change (last/first)	Groups				
	Control	Pb	Hg	Cd	Al
Latency, negative wave	0.989±0.007	1.161*±0.075	0.935±0.061	0.957 ±0.035	1.062±0.025
Latency, positive wave	0.995±0.009	0.965 ±0.024	1.067±0.401	1.084 ±0.039	1.008±0.046
Amplitude, negative wave	0.856±0.221	0.659*±0.243	0.809±0.285	0.752*±0.214	0.841±0.244
Amplitude, positive wave	0.895±0.182	0.795 ±0.233	0.872±0.462	0.853 ±0.372	0.972±0.659
Amplitude, summed	0.849±0.198	0.746*±0.194	0.841±0.349	0.804 ±0.252	0.889±0.352

Visual evoked potential

Relative change (last/first)	Groups				
	Control	Pb	Hg	Cd	Al
Latency, main wave	0.975±0.010	1.083 ±0.085	1.005±0.057	1.107±0.065	1.042±0.025
Amplitude, main wave	0.839±0.176	0.736*±0.265	0.798±0.257	0.802±0.244	0.921±0.251

A measurable decrease in the amplitude between the first and the last evoked potentials was seen in each animal, control or treated, in both modalities. This is indicated by values below 1.00 in *Table 1*. Control rats showed merely a moderate decrease of the evoked potential amplitude. Most of the above-mentioned metals,

however, induced a much stronger decrease in both the somatosensory and the visual evoked potentials (in the former, mainly when calculated for the negative [upward] peak or for the peak-to-peak amplitude of the main waves).

We tested the frequency dependence, up to now, only on the somatosensory evoked potentials. The influence of stimulation frequency on the onset latency and duration of the potentials is shown in *Table 2*. As one can see, only duration proved to be frequency-dependent and this dependence was significantly influenced by lead but only marginally by aluminum.

TABLE 2. Group averages of parameters of the somatosensory evoked potential, demonstrating frequency dependence

Stimulation frequency	Onset latency (ms)			Duration (ms)		
	Control	Pb	Al	Control	Pb	Al
1 Hz	7.73±0.38	8.15±0.43	8.02±0.51	10.73±1.22	12.10 ±1.21	11.64±1.18
2 Hz	7.96±0.57	8.23±0.37	8.18±0.33	12.65±1.37	13.58 ±1.15	12.82±1.56
5 Hz	7.75±0.51	8.29±0.41	7.90±0.35	12.55±1.66	15.07*±2.12	13.48±1.11

DISCUSSION

The present way of evaluation of numeric parameters of sensory evoked potentials has shown that they can be used for detecting toxic effects. In humans, alterations of visual evoked potential latency on mercury exposure was described by Urban et al. (1996) who also stressed the sensitivity of such investigations in detecting toxic effects. Otto et al. (1981) reported about alterations of the human auditory evoked potential on lead exposure, and Lilienthal et al. (1996) found its analogue in monkeys. Similar effects of other toxicants, e.g. ozone in rats (Custodio-Ramirez and Paz, 1997), were also described.

Various forms of evoked activity, including sensory potentials, are routinely recorded also in humans. As a number of data in the literature indicate the sensitivity of such evoked activities to toxic influence, these can be promising candidates for functional biomarkers of neurotoxic effect.

REFERENCES

ARCHER, T. (1993). "Behavioral retardation in the neuropathology of mental retardation." Acta Pathol. Microbiol. Immunol. Scand. 40:35–56.

BELLINGER, D., LEVITON, A., WATERNAUX, C., NEEDLEMAN, H., and RABINOWITZ, M. (1989). "Low-level lead exposure, social class and infant development." Neurotox. Teratol. 10:479–503.

CUSTODIO-RAMÍREZ, V. and PAZ, C. (1997). "Ozone produces functional deficits in the rat visual pathway." Electroenceph. Clin. Neurophysiol. 104:269–273.

DEVI, M. and FINGERMAN, M. T. I. (1995). "Inhibition of acetylcholinesterase activity in the central nervous system of the red swamp crayfish, Procambarus clarkii, by mercury, cadmium, and lead." Bull. Environ. Contam. Toxicol. 55:746–750.

FERN, R., BLACK, J. A., RANSOM, R. R., and WAXMAN, S. G. T. I. (1996). "Cd(2+)-induced injury in CNS white matter." J. Neurophysiol. 76:3264–3273.

Flora, S. J. and TANDON, S. K. (1987). "Effect of combined exposure to cadmium and ethanol on regional brain biogenic amine levels in the rat." Biochem. Int. 15:863–871.

FRANCESCHETTI, S., BUGIANI, O., PANZICA, F., TAGLIAVINI, F., and AVANZINI, G. (1990). "Changes in excitability of CA1 pyramidal neurons in slices prepared from $AlCl_3$-treated rabbits." Epilepsy Res. 6:39–48.

GRANDJEAN, P., BROWN, S. S., REAVEY, P., and YOUNG, D. S. (1994). "Biomarkers of chemical exposure: State of the art." Clin. Chem. 40:1360–1362.

HOSOVSKI, E., MASTELICA, Z., SUNDERIC, D., and RADULOVIC. D (1990). "Mental abilities of workers exposed to aluminum." Med. Lav. 81:119–123.

KUMAR, M. V. and DESIRAJU, T. (1992). "EEG spectral power reduction and learning disability in rats exposed to lead through postnatal developing age." Ind. J. Physiol. Pharm. 36:15–20.

KUMAR, R., AGARWAL, A. K. and SETH, P. K. T. I. (1996). "Oxidative stress-mediated neurotoxicity of cadmium." Toxicol. Lett. 89:65–69.

LILIENTHAL, H. and WINNEKE, G. (1996). "Lead effects on the brain stem auditory evoked potential in monkeys during and after the treatment phase." Neurotox. Teratol. 18:17–32.

LILLE, F., HAZEMAN, P., GARNIER, R., and DALLY, S. (1988). "Effects of lead and mercury intoxication on evoked potentials." Clin. Toxicol. 26:103–116.

MOHD, M. A., MURTHY, R. C. and CHANDRA, S. V. (1986). "Developmental and long-term neurobehavioral toxicity of low-level in utero Cd exposure in rats." Toxicol. Teratol. 8:463.

MOLFESE, D. L., LAUGHLIN, N. K., MORSE, P. A., LINNVILLE, S. E., WETZEL, W. F., and ERWIN, R. J. (1986). "Neuroelectrical correlates of categorical perception for place of articulation in normal and lead-treated rhesus monkeys." J. Clin. Exp. Neuropsychol. 8:680–696.

NAGYMAJTÉNYI, L., PAPP, A. and DÉSI, I. (1997a). "Experimental neurotoxic effect of simultaneous dimethoate and heavy metal (Hg, Cd) treatment." In: Proceedings of the 4th International Workshop on Clinical Toxicology, Bratislava, pp. 157–170.

NAGYMAJTÉNYI, L., SCHULZ, H., PAPP, A., and DÉSI, I. (1997b). "Behavioral and electrophysiological changes caused by subchronic lead exposure in rats." Centr. Eur. J. Occup. Environ. Med. 3:195–209.

NEEDLEMAN, H. L. (1983). "Lead at low dose and the behavior of children." Acta Psychiatr. Scand. 303:26–37.

NEEDLEMAN, H. L. and GATSONIS, C. A. (1990). "Low-level lead exposure and the IQ of children." JAMA 263:673–678.

OTTO, D. BENIGNUS, V., MULLER, K., and BARTON, C. (1981). "Effects of age and body lead burden on CNS function in young children. I. Slow cortical potentials." Electroenceph. Clin. Neurophys. 52:229–239.

OTTO, D., ROBINSON, G., BAUMANN, S., SCHROEDER, S., MUSHAK, P., KLEINBAUM, D., and BOONE, L. (1985). "5-year follow-up study of children with low-to-moderate lead absorption: electrophysiological evaluation." Environ. Res. 38:168–186.

RICE, D. (1996). "Sensory and cognitive effects of developmental methylmercury exposure in monkeys, and a comparison to effects in rodents." Neurotoxicology 17:139–154.

RUPPERT, P. H., DEAN, K. F. and REITER, L. W. (1985). "Development of locomotor activity of rat pups exposed to heavy metals." Toxicol. Appl. Pharmacol. 78:69–77.

SCHREEDER, M. T., FAVERO, M. S., HUGHES, J. R., PETERSEN, N. J., BENNETT, P. H., and MAYNARD, J. E. (1983). "Dialysis encephalopathy and aluminum exposure." J. Chronic Dis. 36:581–593.

SCHULZ, H., NAGYMAJTÉNYI, L., PAPP, A., and DÉSI, I. (1997). "Behavioral and neurophysiological consequences of subchronic mercury exposure in rats." Centr. Eur. J. Occup. Environ. Med. 3:210–223.

SHUKLA, G. S., HUSSAIN, T. and CHANDRA, S. V. T. I. (1987). "Possible role of regional superoxide dismutase activity and lipid peroxide levels in cadmium neurotoxicity: in vivo and in vitro studies in growing rats." Life Sci. 41:2215–2221.

SINGER, R., VALCIUKAS, J. A. and ROSENMAN, K. D. (1987). "Peripheral neurotoxicity in workers exposed to inorganic mercury compounds." Arch. Environ. Health 42:181.

SMITH, M. J., PIHL, R. O. and FARRELL, B. T. I. (1985). "Long term effects of early cadmium exposure on locomotor activity in the rat." Neurobehav. Toxicol. Teratol. 7:19–22.

SOLEO, L., URBANO, M. L. and AMBROSI, L. (1990). "Effects of low exposure to inorganic mercury on psychological performance." Brit. J. Ind. Med. 47:105–109.

SOLOMON, P. R., KOOTA, D. and PENDLEBURY, W. W. (1990). "Disrupted retention of the classically conditioned nictitating membrane response in the aluminum-intoxicated rabbit using electrical stimulation of the brain as a conditioned stimulus." Neurobiol. Aging 11:523–528.

URBAN, P., LUKÁS, E., BENICKY, L., and MOSCOVICOVA, E. (1996). "Neurological and electrophysiological examination on workers exposed to mercury vapors." Neurotoxicology 20:191–196.

WHO (1991) Mercury, Inorganic. Environmental Health Criteria 118. WHO, Geneva

WHO (1992) Cadmium. Environmental Health Criteria 134. WHO, Geneva

WHO (1995). Inorganic Lead. Environmental Health Criteria 165. WHO, Geneva

WHO (1997). Aluminum. Environmental Health Criteria 194. WHO, Geneva

WINNEKE, G., BROCKHAUS, A., EWERS, U., KRAMER, U., and NEUF, U. (1990). "Results from the multicenter study on lead neurotoxicology in children: implications for risk assessment." Neurotox. Teratol. 12:553–559.

YARGICOGLU, P., AGAR, A., SENTURK, U. K., IZGUT, N., and OGUZ, Y. (1996). "Effect of chronic cadmium exposure on VEP and EEG spectral components." Int. J. Neurosci. 85:173–184.

YUAN, Y. and ATCHISON, W. D. (1994). "Comparative effects of inorganic divalent mercury, methylmercury and phenylmercury on membrane excitability and synaptic transmission of CA1 neurons in hippocampal slices of the rat." Neurotoxicology 15:403–411.