Polish Journal of Occupational Medicine Vol. 3, No. 1, 51-67, 1990

BRAIN ELECTRICAL ACTIVITY (EEG) AFTER REPETITIVE EXPOSURE TO CHLORPHENVINPHOS, AN ORGANOPHOSPHATE ANTICHOLINESTERASE: I. RABBIT

SŁAWOMIR GRALEWICZ, WOJCIECH KOWALCZYK, ROMAN GÓRNY and RENATA SOĆKO

Department of Toxicity Evaluation, The Nofer Institute of Occupational Medicine, Lodz, Poland

Key words: Organophosphate, Cholinesterase activity, Blood, Brain, Hippocampus, EEG, Rabbit

Abstract. Cholinesterase (ChE) activity in blood and brain as well as the hippocampal and cortical EEG were investigated in rabbits exposed once a day for a period of two weeks to an organophosphate insecticide, chlorphenvinphos (CVP). The daily dose of CVP was 14.0 mg/kg i.p. ChE activity in plasma and erythrocytes decreased by 60 and 48%, respectively, by the end of exposure and returned to the preexposure level within 35 days. In the exposed animals, killed and dissected a day after, the ChE activity in blood had been found normal, the level of ChE activity in some parts of the brain was still significantly depressed. The spontaneous hippocampal EEG showed no changes as soon as 24 hours after the exposure. However, after a period sufficiently long for the normalization of ChE activity in blood, the hippocampal arousal response (theta rhythm) to click and to a tone associated with pain was found heightened in the exposed rabbits. Moreover, spectral analysis of 5 minute EEG samples revealed a decrease in the content of 7—13 Hz activity in the cortex of the exposed animals as compared to the control ones. The obtained data suggest that exposure to CVP may lead to functional changes in the brain outlasting the period of ChE depression.

INTRODUCTION

Human data suggest that exposure to organophosphorus compounds (OP) — irreversible inhibitors of cholinesterase (ChE) — may result in alteration in brain functions outlasting the depression of ChE activity. Disturbances in psychic functions (15) as well as alterations in EEG (7) have been noted in workers several months or even years after the exposure. The persistence of long-lasting disturbances in EEG have been confirmed in monkeys after a single exposure to sarin, a chemical

Address reprint requests to S. Gralewicz, Department of Toxicity Evaluation, The Nofer Institute of Occupational Medicine, P.O. Box 199, 90-950 Lodz, Poland

warfare agent (4). To our knowledge, the possibility that similar effects may be produced by less toxic OP-s used in agriculture has not been tested experimentally.

In previous experiments on rabbits we studied the effects of a single exposure to chlorphenvinphos (Phosphoric acid 2-chloro-1/2, 4-dichlorophenyl/vinyl diethyl ester-CVP), an OP insecticide used widely in Poland for control of the Colorado beettle (5). Apart from a transient increase in the content of the hippocampal rhythmic slow activity (RSA) visual inspection of the records revealed no clear-cut and persisting changes (11). The present work concerns the effects of a repetitive exposure to CVP on hippocampal and neocortical EEG in the same species. Similarly, as in the previous work, special attention has been paid to the hippocampal EEG; at least one form of the hippocampal activity, namely the immobility-related RSA (IRSA) is controlled by the cholinergic system and may serve as an indicator of its functional state (3).

MATERIALS AND METHODS

Animals

Two groups of white New Zealand rabbits, males, about 6 months old and of 3.0-3.5 kg of body weight at the beginning of the experiments have been used. Determinations of ChE activity in blood and in selected parts of the brain were performed in one group of animals ($n = 14$, five exposed and nine controls). The rabbits of the second group ($n = 10$, five exposed and five controls) were prepared for the electrophysiological studies by implanting them with chronic intrabrain electrodes.

The pesticide and exposure conditions

The pesticide was obtained from the manufacturer (ORGANIKA-AZOT, Jaworzno, Poland) in the form of a concentrate containing 92% of CVP (a mixture of cis and trans-isomers at a 1 : 9 ratio) and about 8% of impurities (enolophosphates). Before use it was diluted with warm (40°C) sterile olive oil in order to obtain the required concentration (14.0 mg/ml). CVP was administered intraperitoneally. Each animal was injected ten times in a period of two weeks (one injection a day excluding saturdays and sundays). The daily dose of CVP in the exposed animals was 14 mg/kg which constituted about 64% of the lowest dose used in the previous studies (11). It was expected that at this exposure

level the ChE activity in blood would not decrease below 50% of the control value, i.e. would remain close to the safety limits accepted in some countries (2). The control animals were injected with pure olive oil in volume of 1 ml/kg.

Determination of ChE

The ChE activity in blood (plasma and erythrocytes) was determined in blood samples taken from the punctured ear marginal vein. In the exposed animals the determinations were made at the following time points: before the first CVP injection, 3 hrs after the first injection, 10 min before the tenth injection, 3 hrs, 24 hrs, 48 hrs, 72 hrs, 7 days after the last injection and then once a week till the return of ChE activity to at least 90% of the control value. When it had happened the exposed animals as well as the unexposed ones were decapitated, the brains were removed from skulls as quickly as possible and dissected on ice into eight parts: cerebellum, medulla oblongata, midbrain, diencephalon, hippocampus, entorhinal cortex, temporo-parietal cortex and anterior part of the cerebral hemisphere (including white matter and the head of the caudate nucleus). Each part was carefully weighed and homogenized with addition of cold Sorenson buffer. Voss and Sachsee method (16) was applied for determination of the ChE activity in blood and in brain homogenates.

Surgery

All animals used for electrophysiological experiments were implanted stereotaxically with chronic bipolar electrodes under barbiturate anaesthesia (hexobarbital sodium). The electrodes were made of teflon coated stainless steel wire of 0.2 mm in diam (Medwire). The tips of the electrodes were placed in the dorsal hippocampus (4 mm posteriorly to Bregma, 4 mm laterally to the midline, and 4 mm below the brain surface), the anterior neocortex (5 mm anteriorly to Bregma, 3 mm laterally to the midline), and the posterior neocortex (9 mm posteriorly to Bregma and 6 mm laterally to the midline). The details of the surgery have been described in previous papers (10, 11). The animals were allowed four to six weeks recovery after surgery.

Apparatus

EEG recordings were made with the use of an 8-channel electroencephalograph. Apart from the EEG motor activity was recorded on

one of the channels with the use of the "free cable" method. The outputs of the EEG amplifiers were connected through an AD converter with an IBM microcomputer. In some tests the EEG signals were recorded simultaneously on paper and in the mass memory of the computer. During the recordings the animals remained in an observation cage (120×60×60 cm) with transparent roof and front and with a grid floor. The cage was located in a semi-soundproof enclosure. A closed-circuit TV system enabled the experimenter to watch the animal behavior during the recording.

Procedure of the electrophysiological testings

The procedure of the electrophysiological testing consisted of:

a) Test 1 — recording of the spontaneous hippocampal and neocortical EEG with no sporadic stimuli applied.

Each recording session consisted of three 5 min periods of continuous EEG recording separated by 5 min intervals. Before the exposure three recording sessions were performed at one week intervals. After the exposure, sessions of recording the spontaneous EEG were performed 24 hrs, 7 days and 14 days after the last CVP injection;

b) Test 2 — testing the magnitude of the hippocampal arousal in response to a strong acoustic stimulus.

During this test animal remained seated within a tight enclosure separated by a thin plywood partition from the rest of the observation cage. Owing to this the distance between the loudspeaker (located within the observation cage) and the animal's ears remained relatively constant during the testing. The testing consisted of a repetitive (30×) application of a strong auditory stimulus (click). The stimulus was applied only when LIA (large irregular activity) was present in the hippocampus, and at intervals no shorter than 10 sec. The magnitude of the hippocampal electroencephalographic arousal was estimated on the basis of the duration of the RSA train induced by each click (Fig. 1). This testing was performed before the exposure once and was repeated eight weeks after the last injection of CVP;

c) Test 3 — testing the hippocampal and neocortial EEG before and after a change of the biological significance of a sporadic stimulus in the experimental situation.

This test was performed once on days 49, 50 and 51 after the exposure. It consisted of three 15 min sessions separated by a 24 hour interval. Each session was composed of three 5 min periods. During the first session spontaneous EEG was recorded for 5 min (period I), then an acoustic stimulus (a 1000 Hz tone of 10 s duration) was applied ten

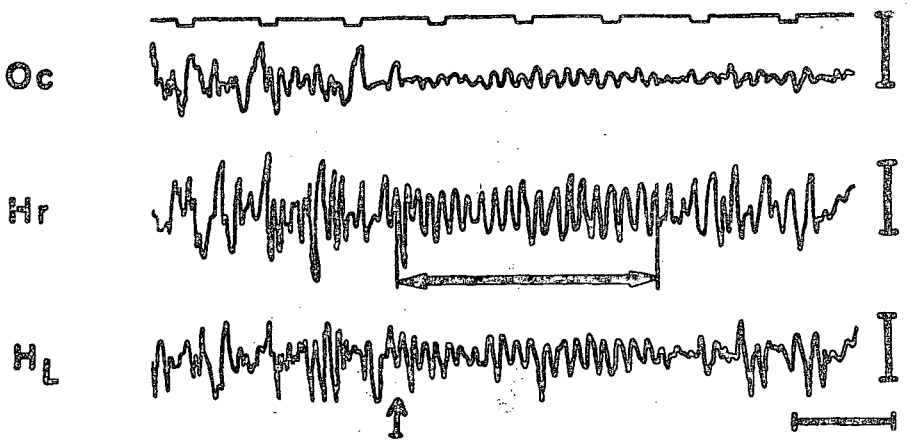

Fig. 1. A section of EEG record showing the hippocampal electroencephalographic arousal response to the click. Denotations of traces: Oc - occipital cortex, Hr — right hippocampus, Hl — left hippocampus. The upward directed arrow marks the moment of the click application. The double horizontal arrow marks the beginning and the end of the arousal response. Calibration: 200μV, 1 sec.

times at 1 min intervals (period II and III). During the second session in periods II and III a short (0.1 s) electric footshock (sine wave 50 Hz, 1.5 mA) was applied at 1.0 Hz frequency during the last 5 secs of the tone duration. Thus, the tone became a signal of unavoidable footshock. During the third session the procedure was exactly the same as during the first one. During the first and third session EEG was recorded continuously on paper and in the computer mass memory. Records were not made during the second session.

Data analysis

The ink written EEG records were analysed visually. In the hippocampal spontaneous EEG (Test 1 and partially Test 3) the content of the two basic patterns of activity — LIA and RSA — were evaluated. Within RSA, the movement-related RSA (MRSA) and the immobility--related RSA (IRSA) were estimated separately on the basis of the RSA frequency and the presence of motor activity (Fig. 2). Records stored on discs (Test 3) were subjected to a spectral analysis by means of a computer program developed for this purpose in our laboratory.

Statistics

Statistical analysis was performed with the use of a two-way ANOVA and Tukey test (17). Data from each session (between-group

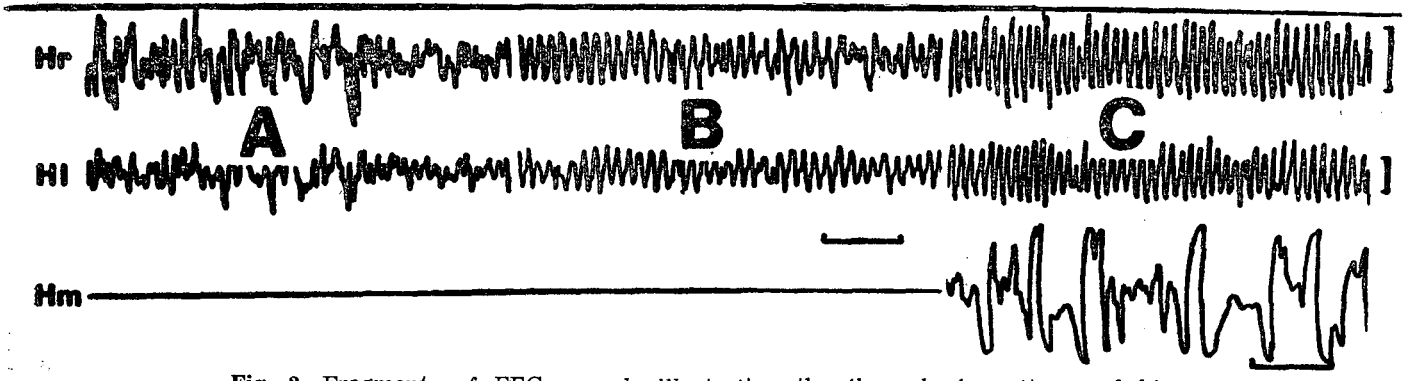

Fig. 2. Fragments of EEG records illustrating the three basic patterns of hippocampal activity. A — large irregular activity (LIA), B — immobility-related rhythmic slow activity (IRSA), C — movement-related rhythmic slow activity (MRSA). Denotations: Hr — right hippocampus, Hl — left hippocampus, Hm — head movements, Calibration: 200 μV, 1 sec.

comparisons) and from each group (within-group comparisons) were analysed separately.

RESULTS

General observations

No changes in general behavior in home cages were observed in the exposed animals during the period of exposure. However, in all exposed animals injections of CVP resulted in a 5—8% decrease in body weight (6.1% on the average) at the end of the exposure. It returned to the preexposure level within one week after the exposure.

Injections of CVP resulted in a decrease in the rectal temperature. This decrease attained maximum (0.8—2.1°C, 1.6°C on the average) within 90—120 min after the injection and disappeared within 24 hrs. The effect of successive injections became gradually smaller, failing finally to appear.

Blood ChE activity during and after the exposure

Figure 3 presents changes in the ChE activity in full blood, plasma and erythrocytes in the course and after the exposure to CVP. A two-way ANOVA (Compartment×Time) showed that the effect of Time was highly significant ($F_{2,12} = 76.55$, $p < 0.0001$). The degree of ChE depression as well as the rate of normalization of the enzyme activity differed depending on the compartment tested (effect of Compartment: $F_{2,12} = 12.83$, $p < 0.002$, Time × Compartment interaction: $F_{2,12} = 11.54$, $p < 0.002$). The erythrocyte ChE appeared to be significantly more sensitive to CVP than the plasma ChE. The plasma ChE activity was decreased by about 50% by the end of the exposure, whereas the activity of the erythrocyte ChE was reduced by about 60% on the average. The rate of normalization of activity was slower in the case of the erythrocyte ChE. In both these compartments ChE activity returned to 90—100% of the preexposure level within 28 days in three animals, and 35 days in the remaining two of this group.

Brain ChE activity

In the exposed animals, killed and dissected a day after the ChE activity in plasma and erythrocytes had returned to no less than 90% of the pre-exposure value, the level of ChE activity in some parts of the brain, namely in the midbrain, diencephalon, hippocampus, entor-

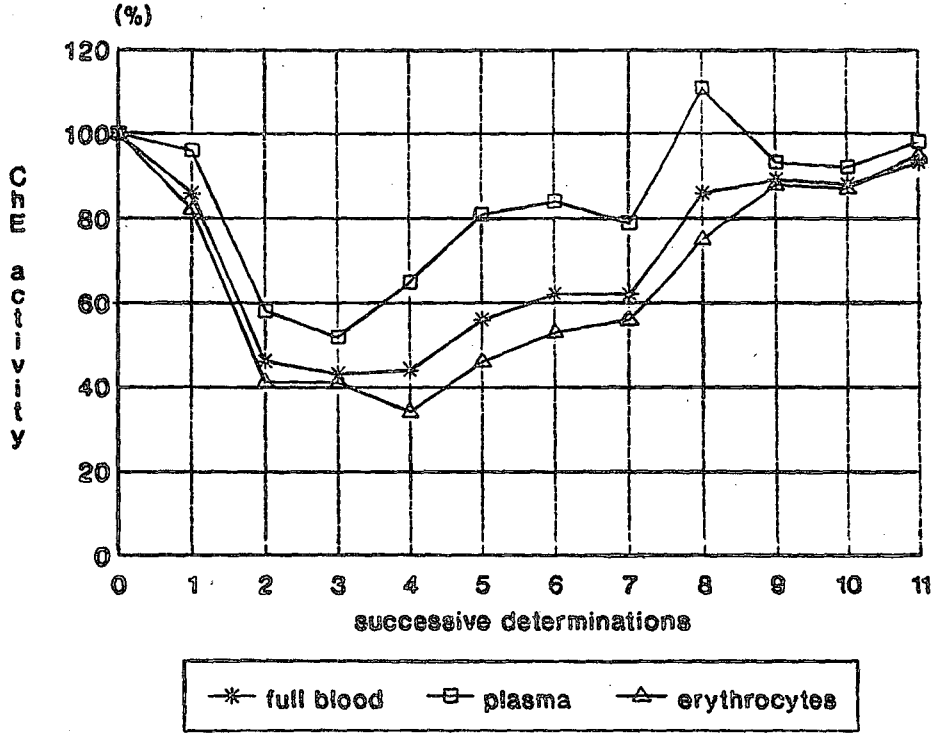

Fig. 3. Blood ChE activity in rabbits during the course of and after repetitive exposure to chlorphenvinphos. Ordinate — ChE activity as a percent of the pre-exposure value. Abscissa — successive determinations; 0 — control, 1 — three hours after the first injection, 2—10 min before the tenth injection, 3, 4, 5, 6, 7, 8, 9, 10, 11 — three hours, one day, two days, three days, one week, three weeks, four weeks and five weeks after the tenth injection, respectively.

hinal cortex and temporo-occipital cortex was significantly lower than in the unexposed (control) rabbits (Table 1).

Brain electrical activity

a) Spontaneous hippocampal EEG before and after exposure to CVP (Test 1).

No between-group (exposed-vs-control) differences were found with respect to the global content of the three basic patterns of hippocampal activity, i.e. LIA, IRSA and MRSA. The only change seen in this test was a gradual decline in the MRSA content after the exposure. However, the groups did not differ in this respect;

b) Magnitude of the hippocampal arousal in response to click before and after the exposure (Test 2).

TABLE 1. A comparison between the brain ChE activity in normal rabbits and in rabbits exposed repetitively to chlorphenvinphos in the past. In the exposed rabbits brain ChE was determined a day after the ChE activity in plasma and erythrocytes had been found above 90% of the preexposure values.

brain region	ChE activity * (mean and SE)		statistics (Tukey test)
	control animals n = 9	exposed animals n = 5	
cerebellum	22.04 (0.39)	20.89 (0.92)	NS
medulla oblongata	21.55 (0.79)	18.51 (1.24)	NS
midbrain	29.30 (1.05)	24.59 (1.60)	$p < 0.05$
diencephalon	18.97 (0.34)	16.19 (0.76)	$p < 0.01$
hippocampus	19.93 (0.81)	15.25 (1.02)	$p < 0.01$
pyriform lobe	15.12 (1.01)	9.55 (0.82)	$p < 0.01$
temporo-occipital cortex	19.47 (1.26)	14.37 (1.60)	$p < 0.05$
frontal pole	35.45 (1.95)	29.01 (2.86)	NS

* ChE activity is expressed in number of micromoles of acetyltiocholine hydrolysed by one gram of fresh brain tissue during one min at 30°C.

In order to analyse the changes in the RSA response to click, both sessions (one before and one after the exposure) were divided into blocks of five trials each. For each block the mean duration of the RSA response to click was calculated. In both groups and in both sessions the RSA response shortened gradually (Fig. 4), i.e. habituation occurred. In the control group the magnitude of the RSA response as well as the habituation rate was similar in both sessions. In the exposed group the habituation proceeded at a similar rate in both sessions but the magnitude of the response after the exposure was generally higher (i.e. RSA persisted longer) than before the exposure in the same group ($F_{(1,8)} = 14.5$, $p < 0.01$), as well as in the control group after the exposure ($F_{(1,8)} = 9.94$, $p < 0.02$);

c) Effect of exposure on the hippocampal and neocortical EEG before and after a change in biological significance of stimuli in the experimental situation (Test 3).

For the purpose of the analysis, each of the 15 min records made during the first (control) and during the third session were separated into three 5 min sections. Section 1 was from the control period (with no sporadic stimulus applied). Sections 2 and 3 encompassed the period

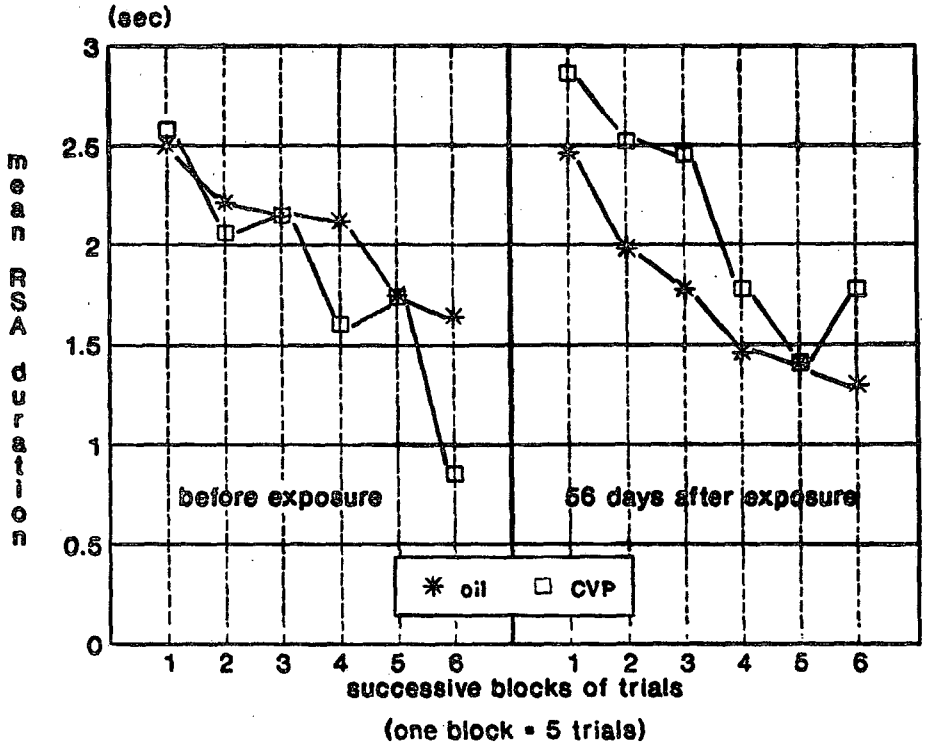

Fig. 4. The magnitude of the hippocampal electroencephalographic arousal response to the click before and 56 days after the repetitive exposure to chlorphenvinphos in rabbits.

during which the acoustic stimulus (1000 Hz tone) was presented every one min. The ink — written records were analysed visually and the digital records were subjected to spectral analysis.

In all animals the presentation of the 1000 Hz tone resulted in the appearance of RSA in the hippocampal EEG. In the exposed group, however, this effect was significantly larger (the trains of RSA were longer) than that in the control. The differences between groups were particularly strong during the third session ($F_{(1,8)} = 25.83$, $p < 0.001$). Similarly as in the case of the response to click the trains of RSA shortened during successive presentations of the tone but the groups did not differ in this respect (interaction was not significant, Fig. 5).

The results of the spectral analysis of the 5 min samples of the hippocampal EEG are presented in Fig. 6. Statistical comparisons of the spectral power within the 1—4 Hz, 4—7 Hz and 7—9 Hz bands revealed no significant differences. The analysis of electrocorticograms, however, revealed that in the exposed rabbits in the first and third

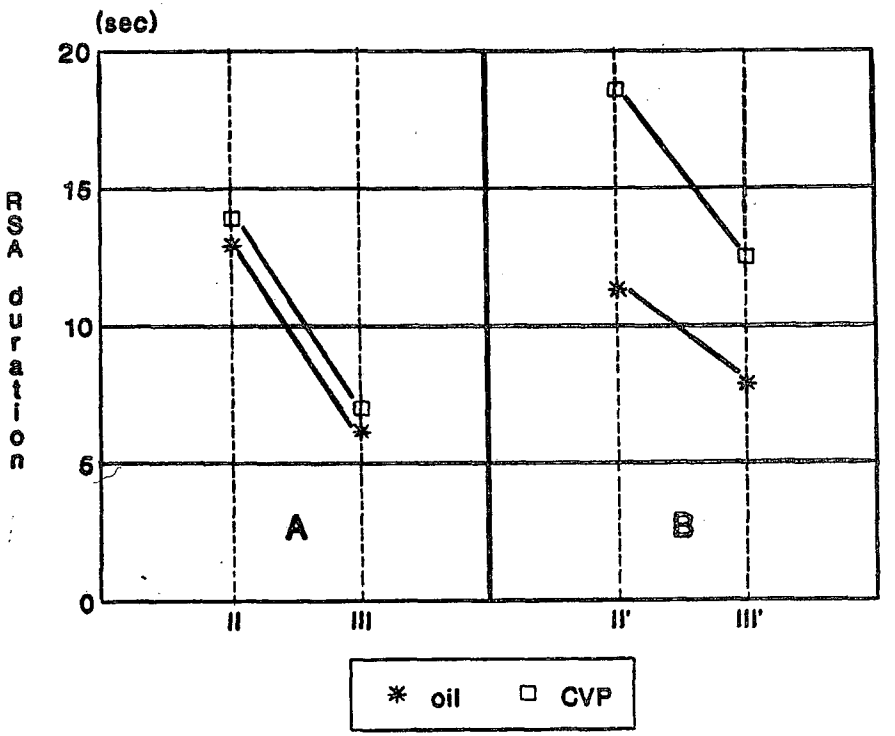

Fig. 5. The magnitude of the hippocampal electroencephalographic arousal response to the 1000 Hz tone before (A) and after (B) its association with an unavoidable footshock. The test was performed on days 41—43 after the repetitive exposure to chlorphenvinphos. Ordinates: duration of the RSA train (in seconds) measured from the start of the tone. Abscissa: Roman numerals denote successive 5 min recording periods of the 15 min session during which the tone was presented. Numerals followed by apostrophe refer to periods of the third session (after the conditioning). See text for further explanations.

session the proportion of activity within the 1—4 Hz band was higher (the differences were approaching statistical significance) and that within the 7—13 Hz band was lower than in the control animals (first session: $F_{(1,8)}=5.75$, $p<0.05$; third session: $F_{1,8}=7.1$, $p<0.05$). No differences between groups as well as between sessions were found with respect to the proportions of electrocortical activity within the remaining frequency bands (4—7 Hz, 13—21 Hz and 21—32 Hz, Fig. 7).

DISCUSSION

Two facts discovered in the present studies require consideration. Firstly, in rabbits exposed repetitively to CVP the activity of ChE in blood, particularly in plasma, returns to a normal (preexposure) level

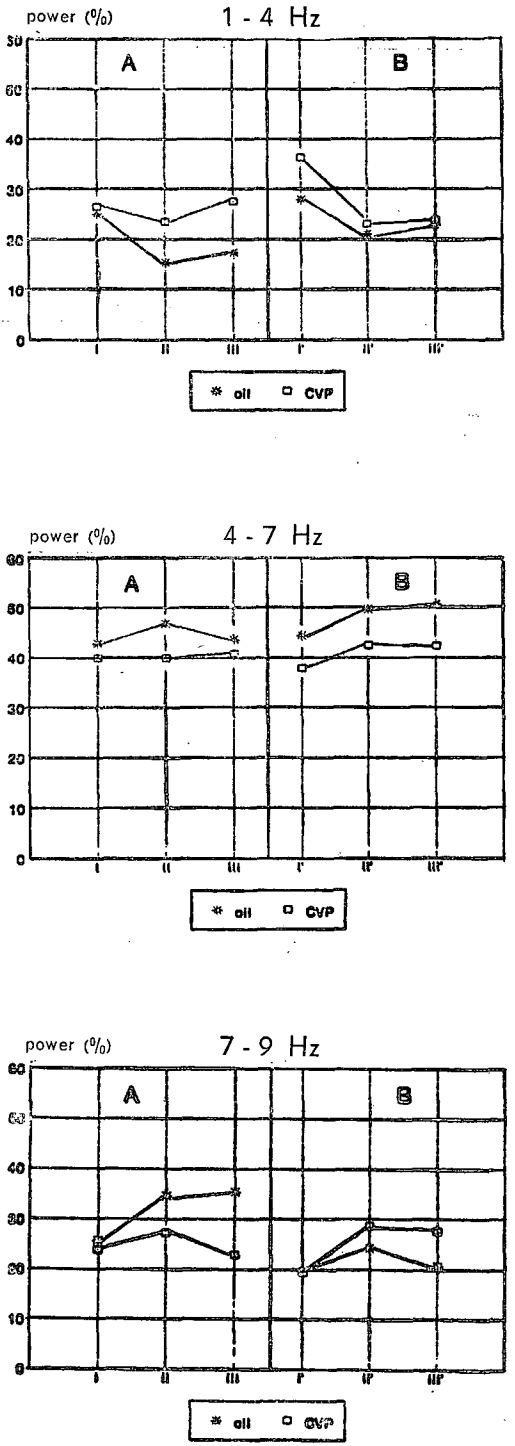

Fig. 6. Effect of the change on biological significance of the experimental situation on the distribution of power in the spectrum of the hippocampal EEG in the control rabbits and in rabbits exposed repetitively to chlorphenvinphos. This test was performed on days 49—51 after the exposure. Analysed were 5 min EEG samples recorded during the following periods: I, I' — control periods (no sporadic stimulus applied during the recording), II, III, II' and III' — periods during which the 1000 Hz tone was applied. Left graph (A) — results obtained before the association of the tone with the unavoidable footshock, right graph (B) — results obtained after the association of the tone with the unavoidable footshock (see text for further explanation).

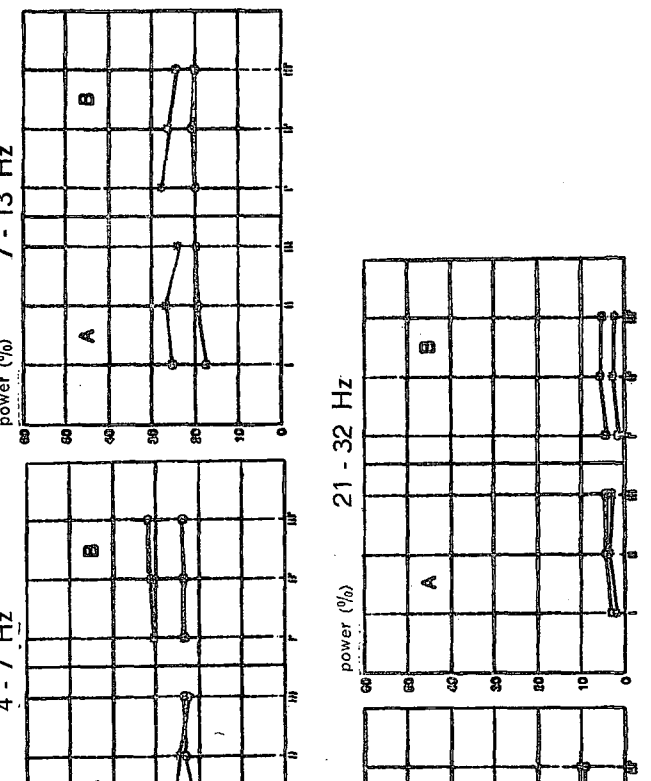

Fig. 7. Effect of the change in biological significance of the experimental situation on the distribution of power in the spectrum of the cortical EEG in the control rabbits and in rabbits exposed repetitively to chlorphenvinphos. This test was performed on days 41—43 after the exposure. Remaining explanations as in Fig. 6.

faster than in some parts of the brain, and secondly, detectable changes in the brain electrical activity exist after normalization of the ChE activity in blood and in the brain.

Blood is the only element in which ChE can be determined in exposed workers and the assessment of the exposure level as well as the degree of recovery after the exposure is usually based on the outcome of this determination. When the level of ChE inhibition in plasma and erythrocytes differs from that in other parts of the body, especially in the brain, the risk of diagnosing an intoxicated person healthy and capable to work is high.

So far direct data which could make possible prediction of the level of ChE inhibition as well as the normalization rate in the human brain after CVP exposure (as well as after exposure to other OP-s) are lacking. Relationships which may exist in man can only be extrapolated from the results of experiments performed on animal models. The situation is more complicated as the relationship may differ depending on the animal species (13) as well as on the compound studies (9, 14). It means that in order to obtain information, on which a reliable diagnosis could be based, data from several species concerning the same OP are necessary.

The lack of significant changes in the hippocampal spontaneous EEG as early as 24 hrs after exposure (when the ChE activity was still low) is in agreement with our previous data (11). It may suggest that the part of the brain cholinergic system which is involved in the control of the hippocampal activity (IRSA), similar to that participating in the control of body temperature, can undergo fast adaptive changes. The results of the further tests, however, show that in the exposed animals the functional state of the brain is not exactly the same as in the control ones even eight weeks after the exposure, i.e. after a time sufficient for full normalization of the ChE activity in blood and, most probably, in the brain. It is in accordance with the observations done on exposed workers with histories of exposure to sarin (7) and on monkeys exposed experimentally to the same compound (4). Our data suggest that long-lasting changes may appear also as consequence of exposure to less toxic OP-s, used extensively as agricultural pesticides.

The longer duration of electroencephalographic arousal in the hippocampus (RSA) in response to the click as well as to the tone after its association with pain, suggests an increased reactivity of sensory and/or emotional systems after a CVP exposure. On the other hand, the slowing of cortical activity, revealed by spectral analysis, suggests a decreased level of unspecific activation during the intervals between successive stimuli. Taken together, these changes may be regarded as

evidence of increased liability of the CNS functional tone after the CVP exposure. Since, in the present experiments, ChE determinations did not parallel the electrophysiological testing, one cannot tell whether the above changes were related to the still diminished ChE activity in some parts of the brain or to long-lasting ChE depression in the past.

It is worth emphasizing here that the changes in the cortical EEG (increased activity within 1—4 Hz band), seen in our exposed rabbits, were opposite to those reported in workers and in monkeys (4, 9) after exposure to sarin; in the latter cases an increased high-frequency activity was observed. It is possible that these differences might be due to some differences between these two OP-s in the effect on other neurotransmitter systems (6) or in the interaction with cholinergic receptors. The ability to interact with postsynaptic cholinergic receptors has been shown in the case of several OP-s (1, 8). According to our knowledge CVP has not been tested for its possible postsynaptic action. In our previous experiments on rats (12) we found that changes in the hippocampal EEG produced by acute CVP exposure were disproportionally weak in comparison to those produced by physostigmine in spite of comparable effects on ChE activity. It may either confirm that physostigmine acts as an agonist of the postsynaptic cholinergic receptors (1) or suggest that CVP interacts with these receptors in an antagonistic fashion.

CONCLUSIONS

1. In rabbits after repetitive exposure to CVP (14.0 mg/kg i.p. daily for two weeks) cholinesterase activity in the blood normalizes faster than in some parts of the brain.

2. In the exposed rabbits, in the absence of strong sporadic stimuli, especially those associated with pain, hippocampal EEG shows no changes as soon as 24 hrs after the end of the exposure.

3. Repetitive exposure to CVP results in an increase of the hippocampal arousal (theta rhythm or RSA) in response to strong acoustic stimuli and to stimuli associated with pain as well as in a decrease of the content of 7—13 Hz activity in the neocortex. These effects are detectable after time sufficient for normalization of the ChE activity in blood.

ACKNOWLEDGEMENTS

This work has been supported by the CPBR grant 11.11 Occupational Medicine.

REFERENCES

1. Albuquerque, E.X., Arakava, Y., Iydris, M., Iydris, M., Schonenberg, B., Desphande, S.S. Activation and blockade of the nicotinic and glutamatergic synapse by reversible and irreversible cholinestease inhibitors. In: Neurobiology of Acetylcholine eds, N.J. Dunn and R.L. Perlman, Plenum Press, New York and London, pp. 301—328, 1987.
2. Ames, R. G., Brown, S.K., Mengle, D.C., Kahn, E., Straton, J.W., Jackson R.J. Cholinesterase activity depression among California agricultural pesticide applicators Am J Industr Med, 15, 143—150, 1989.
3. Bland, B.H. The physiology and pharmacology of hippocampal formation theta rhythms. In: Progress in Neurobiology, 26, 1—54, 1986.
4. Burchfiel, J.L., Duffy, F.H., Sim, V.M. Persistent effects of sarin and dielfrin upon the primate electroencephalogram. Toxicol Appl Pharmacol, 35, 363—379, 1976.
5. Chlorphenvinphos — a rationale for the proposed limits of occupational exposure, a document prepared by the Group of Experts for Chemical Factors, Interdepartmental Committee for Actualization of MAC Values, Poland, 1986 (in Polish).
6. Coudray-Lucas, C., Le Guen, A., Prioux-Goquonneau, M., Cohen, Y., Wepierre, J. Changes in brain monoamine content and metabolism induced by praxon and soman intoxication. Effect of atropine. Xenobiotica, 9, 1131—1138, 1987.
7. Duffy, F.H., Burchfiel, J.L., Bartels, P.H., Gaon, M., Sim. V.M. Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol Appl Pharmacol, 47 161—176, 1979.
8. Eldefravi, A.T., Mansour, N.A., Shaker, N., Abassy, M.A., Eldefravi, M.E. Insecticides affecting acetylcholine receptor interaction. In: Differential Toxicities of Insecticides and Halogenated Aromatics, ed F. Matsumara, Pergamon Press, pp. 401—422, 1984.
9. Farage-Elawar, M., Francis, M. The effect of three organophosphorus esters in blood neurotoxic esterase and acetylcholinesterase, Pesticide Biochem Physiol, 31, 175—181, 1988.
10. Gralewicz S., Gralewicz, K. Changes in hippocampal and cortical EEG after intraventricular administration of cholinolytics in rabbit and cat. Acta Neurobiol Exp, 48: 323—334, 1988.
11. Gralewicz, S., Tomas, T., Górny, R., Kowalczyk, W., Soćko, R. Changes in some physiological and electrophysiological parameters in rabbits after single exposure to chlorphenvinphos. Pol J Occup Med 1989 (in press).
12. Gralewicz, S., Tomas, T., Soćko, R. Effects of single exposure to chlorphenvinphos, an organophosphate insecticide, on electrical activity (EEG) of the rat brain, Pol J Occup Med 1989 (in press).
13. Hutson, D.H., Hathway, D.E. Toxic effects of chlorphenvinphos in dogs and rats. Biochem Pharmacol, 16, 949—962, 1967.
14. Montz, W.E., Kirkpatrick, R.L. Temporal pattern of brain cholinesterase activities of white-footed mice (Peromyscus leucopus) following dosing with diazinon or parathion. Arch Environ Contam Toxicol, 14, 19—24, 1985.
15. Savage, E.P., Keefe, T.J., Mounce, L.M., Heaton, R.K., Lewis, J.A., Burcar, P.J. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch Environ Health, 43, 38—45, 1988.
16. Voss, G., Sachsse, K. Red cell and plasma cholinesterase activities in microsamples of human and animal blood determined by a modified acetyltiocholine (DTNB) procedure. Toxicol App Pharmacol, 16, 764—772, 1970.

17. Winer, B.J. Statistical Principles in Experimental Design, Mc Graw Hill Book Company, New York. 1962.

Received for publication: March 20, 1990
Accepted for publication: March 31, 1990