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EFFECTS OF SINGLE EXPOSURE TO CHLORPHENVINPHOS, AN ORGANOPHOSPHATE INSECTICIDE, ON ELECTRICAL ACTIVITY (EEG) OF THE RAT BRAIN

SŁAWOMIR GRALEWICZ, DSc, PhD, T. TOMAS PhD and RENATA SOĆKO, MSc

Department of Toxicity Evaluation, Nofer's Institute of Occuppational Medicine, Lodz, Poland

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Abstract. The effect of a single exposure to an agricultural insecticide, chlorphenvinphos (CVP), an organophosphorus anticholinesterase, on neocortical seizure activity induced or promoted by cardiazol, and on hippocampal and neocortical EEG was studied in rats. It was found that CVP, given intraperitoneally in doses of 1.0 and 3.0 mg/kg, resulted in no changes in the number and in the duration of epileptic bursts occurring spontaneously, as well as in the content of the hippocampal theta rhythm. The effect of cardiazol (12.5 mg/kg, i.p.) was slightly diminished when the drug was given 3 hours, but not 14 days, after the injection of CVP. I.p. injection of a carbamate cholinesterase inhibitor, physostigmine, in a dose of 1.0 mg/kg resulted in a dramatic increase of the theta content in the hippocampal EEG, and in the total disappearance of the spontaneous seizures. Determination of cholinesterase activity in blood and in the brain in a separate group of subjects showed that after injection of physostigmine (1.0 mg/kg), the inhibition of this enzyme does not exceede the inhibition after injecting CVP in the doses used. It has been suggested that the differences between CVP and physostigmine in their potential to reduce spontaneous epileptic activity and to induce the hippocampal theta rhythm may be due to somewhat antagonistic action of CVP on cholinergic postsynaptic receptors.

INTRODUCTION

The present work is part of a wider study concerning chlorphenvinphos (Phosphoric acid, 2-chloro-1/2,4-dichlorophenyl/vinyl diethyl ester — CVP). Our interest in this organophosphate (OP) arises from the following reasons: i) there is an increasing number of clinical and epidemiological reports showing that even a single exposure to organophosphorus anticholinesterases may result in long-lasting neurobehavioral disturbances (4, 5, 12, 23, 26); ii) OP-s used in agriculture, mainly as

Address reprint requests to S. Gralewicz, Department of Toxicity Evaluation, Nofer's Institute of Occupational Medicine, 8 Teresy Street, 90-950 Lodz, P.O. Box 199, Poland

insecticides, may differ widely with respect to their mammalian toxicity (eg. 18) as well as with respect to some aspects of their mechanism of action (13). It is difficult to tell, then, whether the health effect of exposure to a given OP will be the same as those reported after exposure to another one, unless detailed studies are performed; iii) CVP is being produced and used in Poland in relatively large quantities (7).

In our previous experiments, the effects of a single intraperitoneal exposure to CVP in doses 3.0 mg and 1.0 mg/kg on the blood and brain cholinesterase (ChE) activity and on some forms of behaviour (locomotor and exploratory activity in an open field and response-to-change in "T" maze) were studied in the rat (28). We found that subtle changes in open field behavior might be detected in exposed animals even 12—14 days after the exposure, i.e. after a time sufficient for the normalization of ChE activity in blood and in the brain. The purpose of the present experiments was to assess the influence of a single exposure to CVP at the same doses on some forms of brain bioelectrical activity. Long-lasting changes in EEG, following repeated or even single OP exposure, have been reported in humans (12, 23) as well as in primates (5). According to some reports on rats, EEG is the most sensitive indicator of OP neurotoxicity (9, 10). One might expect then, that changes in the functional state of the brain resulting from exposure to CVP should be more clearly recognizable on the basis of the brain's bioelectrical activity than on the basis of the behavior of the animal after exposure.

In the experiments described below, apart from routine EEG recording, a pharmacological challenge with cardiazol (pentylenetetrazol), an epileptogenic stimulant (21), in a dose sub-threshold for behavioral convulsions but sufficient to produce clear-cut changes in EEG, was used to test brain excitability (14). The broad distribution of cholinergic synapses in the brain (20) suggesting generality of OP influence, the reported possibility of epileptic seizures after an OP exposure (11, 15), as well as the assumed involvement of the cholinergic system in the pathogenesis of epilepsy (24), justified the use of this test.

MATERIAL AND METHOD

Animals. The subjects were male Wistar rats (imp-DaK) 100—180 days old at the start of the experiment and of 200—350 g of body weight. The animals were housed, one to a cage, in standard laboratory conditions (temperature 20—22°C, humidity 50—60%, L/D cycle 12/12 hrs with light on at 6 a.m.), and fed with standard rat chow and water ad libitum. Before the experiments, all the rats were implanted with intrabrain recording electrodes.

Surgery. The surgery was performed under barbiturate anaethesia (penthobarbital sodium, 60 mg/kg, i.p.). Each rat was implanted with one depth bipolar electrode aimed at the dorsal hippocampus (3 mm posterior to Bregma, 1.5 mm lateral from the midline and 3.5 mm below the surface of the skull), and at least one surface electrode placed epidurally over anterior (1.5 mm anterior to Bregma and 2.5 mm lateral from the midline) cortical areas. Details of the surgery and electrode manufacturing were described in a previous work (17). The experiments were initiated 10 days after the surgery.

Apparatus and recording. Brain electrical activity was recorded using an 8-channel electroencephalograph (Beckmann Acutrace) within the 1—35 Hz frequency band. For recording, the animals were put into plastic containers (25×25×40 cm), located in a semi-sound-proof enclosure, and connected to the electroencephalograph with a flexible, low weight and low noise cable. A closed-circuit TV system made it possible to observe the animals' behavior during the experiment.

Drugs. Chlorphenvinfos, technical grade, was obtained from the manufacturer. It was a mixture of cis- and trans- isomers to CVP at 1:8 ratio (92%) and of enolophosphates (8%). Before use, it was dissolved in warm (about 40°C) sterile olive oil, in order to obtain the required concentration. Cardiazol (pentylenetetrazol) was obtained from POLON (Poland) in the form of 10% water solution. Physostigmine was purchased in FERAK GMBH (DDR) in crystalline form. Pyrogen free physiological saline was used to prepare solutions containing cardiazol and physostigmine at required concentration. All the chemicals were administered intraperitoneally in volume 0.8—1.0 ml.

Experimental procedure. The basic experiment consisted of three sessions at 14 day intervals from each other. During the first session, the effect of cardiazol was studied. This session consisted of two recording periods, each of a one hour duration, the first (control period) preceding, and the second, following, the injection of cardiazol. An interval of about 2 minutes, was sufficient to administer the injection. The dose of cardiazol was 12.5 mg/kg. In pilot studies, this dose appeared to be sufficient to provoke bursts of seizure activity in the cortical EEG with no behavioral seizure, or to facilitate markedly the spontaneous bursting (petit mal-like) which occurred in the majority of our animals. During the second session, after the first (control) recording period, some of the animals (High Dose Group — HD) were given CVP in a dose of 3.0 mg/kg, while some (Low Dose Group — LD), CVP in a dose of 1.0 mg/kg, and the remaining (Control Group — C), pure olive oil in the same volume. A three hour recording period followed the CVP (or oil)

injection, after which the animals were given cardiazol, and then the EEG was recorded continuously for a further hour. The procedure during the third session was the same as during the first one. About eight weeks after the exposure to CVP, the rats from the HD group were given physostigmine intraperitoneally in a dose of 1.0 mg/kg. EEG was recorded for one hour before the injection and for another hour immediately following the injection.

ChE determinations. Changes in ChE activity in blood (plasma and erythrocytes) and in selected parts of the brain after exposure to CVP in doses 3.0 and 1.0 mg/kg have been estimated in another studies from this laboratory (35). Therefore, in the present experiments, ChE activity was determined only after an injection of physostigmine and at only one point of time, i.e. 30 min after the administration of the drug. This was performed on eight new male rats of the same stock. The animals were decapitated 25—30 min after the injection of physostigmine ($n = 4$) or physiological saline ($n = 4$). ChE activity in the blood and brain homogenates was determined using the Voss and Sachsee method (32) as previously described (28).

Data analysis. The following data were derived from the obtained electroencephalograms: i) the number and duration of episodes of seizure activity (Fig. 1, A) in the cortical EEG-s in all sessions, ii) the content of high voltage irregular neocortical activity (HVIA), a pattern of activity characteristic for the state of rest and normal sleep (Fig. 1, C), and iii) the content of theta rhythm (Fig. 1, B) in the hippocampal EEG before and after injection of CVP in session II. In sessions I and III the data for analysis (the number and the duration of bursts) were collected from the whole record. The data concerning neocortical (both seizure and HVIA), and hippocampal activity in session II were collected from the one hour control period, from the first and from the third hour after the injection of CVP, and from the one hour recording period after the injection of cardiazol. For the estimation of the content of HVIA in the neocortex and of the content of theta in the hippocampal EEG in session II, a sampling procedure was adopted consisting of the analysis of 5 min samples. The control sample was the last 5 minute section of EEG preceding the injection of CVP (or oil alone), the next were those taken 55—60, 120—125 and 175—180 min, after the injection. For the estimation of the effect of physostigmine, bursts of seizure activity were counted in the whole record. The content of theta in the hippocampal EEG and the content of HVIA in the neocortical records were measured in the last 5 min of the control period and in the sample taken 25—30 min after the injection.

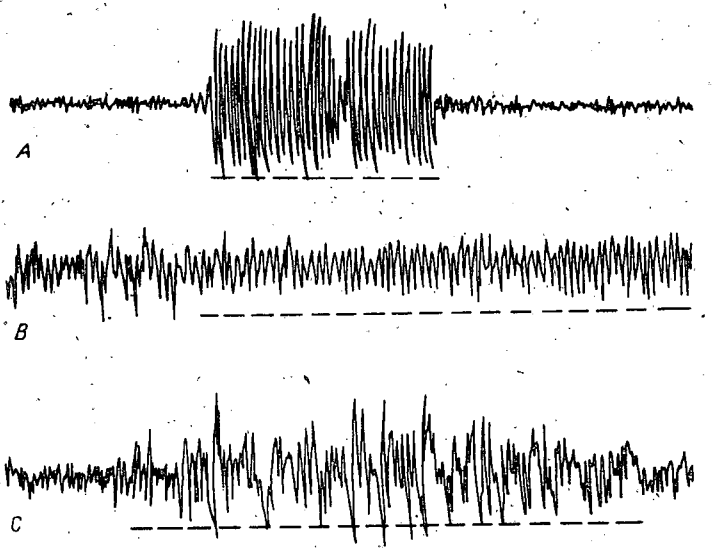

Fig. 1. Sections of EEG records containing the patterns of brain bioelectrical activity subjected to quantitative analysis. (The patterns are underlined with the dashed line). A — a burst of spontaneous seizure activity in the neocortical EEG, B — theta rhythm in the hippocampal EEG, C — high voltage irregular activity (HVIA) in the Neocortical EEG. Callibrations: 1 sec., 500 μV.

Statistics. Statistical evaluation of the data was made by the use of a parametric repeated measures two-way ANOVA and Tukey test (34), the two-way, nonparametric Friedman ANOVA and the sign test and the Wilcoxon test (27).

RESULTS

a) **Changes in cortical activity induced by cardiazol. Influence of CVP.** In fifteen out of the seventeen rats in the present study, bursts of seizure activity of 1—5 sec duration and 8—10 Hz discharge frequency appeared spontaneously in the cortical EEG (Fig. 1A). Similar abnormalities have also been reported by other authors (2, 31). In these animals, 3—5 min after injection of cardiazol (12.5 mg/kg, i.p.), the bursts started to increase in number without overt changes in their duration or in the morphology and frequency of the discharges. This effect attained its peak 15—20 min after the injection and then gradually diminished. In rats with no spontaneous bursts, the effect of cardiazol resembled its effect upon the rabbit (27), and consisted in the appearance of an activity similar to the spontaneous one, but the frequency of discharges might be lower (6—7 Hz).

In order to assess the effects of cardiazol and CVP on the seizure activity, the data from all sessions were subjected to a repeated measures

parametric ANOVA, with groups as Factor A and experimental conditions (periods of recording during successive sessions) as Factor B. The analysis of the number of bursts per hour revealed no significant effect of Factor A ($F(2,14) = 2.03$, N.S.), but the effect of Factor B and AB interaction were statistically significant (effect of Factor B: $F(1,14) = 8.74$, $p < 0.02$, AB interaction: $F(2,14) = 10.21$, $p < 0.002$). Testing of simple effects (Tukey test) revealed significant differences in

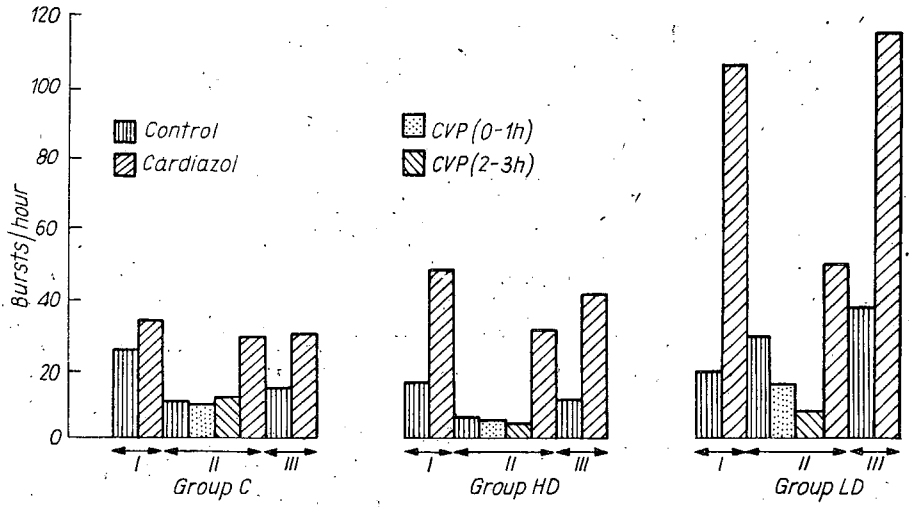

Fig. 2. Diagrams showing the effects of cardiazol on neocortical seizure activity in control rats (Group C), in rats given CVP in a dose of 3.0 mg/kg (Group HD) and in rats given CVP in a dose of 1.0 mg/kg (Group LD). Double arrows underline data obtained during successive sessions (I, II and III) in each group. See text for further explanations.

the number of bursts only within the LD group ($F(7,98) = 11.74$, $p < 0.001$). In this group, the numbers of bursts per hour after injection of cardiazol in session I and III did not differ from each other and were significantly larger than in any of the remaining recording periods ($p < 0.01$ in all cases). The numbers of bursts during periods preceding injections in all sessions and during all recording periods in session II (see Fig. 2) did not differ from one other. It should be added that, in general, in rats of the LD group, the bursts of spontaneous seizure activity appeared more frequently, which may be due to some differences in age at the time of testing (2, 31).

The inability of the parametric ANOVA to reveal differences in the number of bursts per hour between successive recording periods in the remaining groups was due to the large individual variability of this measure. Therefore, the data from group C and group HD were additio-

nally subjected to a nonparametric (Friedman) ANOVA. This analysis revealed significant differences between successive recording periods in group C ($x^2 = 20.1$, $df = 7$, $p < 0.01$) as well as in group HD ($x^2 = 18.3$, $df = 7$, $p < 0.02$). Detailed comparisons revealed that in group C, the numbers of bursts per hour after cardiazol in session I, II and III did not differ from each other and were significantly higher than during remaining recording periods ($p < 0.05$ in all comparisons). No differences in the number of bursts were found between the control periods (before injections) of successive sessions, as well as between the control period and the two periods (0—60 min and 120—180 min) after the CVP injection in session II.

In the HD group, the numbers of bursts per hour after injection of cardiazol in sessions I and III did not differ from each other, and were significantly higher than those in the remaining periods ($p < 0.05$ in all comparisons). The number of bursts after cardiazol in session II was significantly higher than in the preceding periods in the same session ($p < 0.05$ in all comparisons) but it did not differ significantly from that in the control period in session I. No other differences were found.

A similar analysis performed on the data concerning the duration of bursts revealed differences neither between groups, nor between successive recording periods. On the other hand, the results of the ana-

TABLE 1. A Comparison of the Percentage (Content) of Theta Rhythm and HVIA in the Hippocampal and Neocortical EEG, Respectively, Before and After Exposure to CVP

	Type of EEG activity	Before exposure	55—60 min after exposure	120—125 min after exposure	175—180 min after exposure
C n=5	theta	6.72 ± 6.05	12.06 ± 11.83	0.4 ± 0.8	3.8 ± 6.2
	HVIA	29.68 ± 36.37	25.44 ± 21.27	48.28 ± 24.47	41.64 ± 25.28
HD n=7	theta	6.00 ± 7.2	2.24 ± 0.54	2.32 ± 1.36	6.44 ± 8.99
	HVIA	6.66 ± 6.24	9.96 ± 4.45	11.68 ± 8.21	26.18 ± 22.04
LD n=5	theta	22.60 ± 11.19	11.54 ± 8.47	13.01 ± 21.13	14.12 ± 14.26
	HVIA	14.84 ± 26.83	17.50 ± 16.23	37.02 ± 23.57	27.47 ± 14.80

TABLE 2. Effect of Physostigmine (1 mg/kg i.p.) on the Content of Theta Rhythm in the Hippocampal EEG and on Neocortical Seizure Activity

N	Content of theta rhythm (%)		Seizure activity (no. bursts/hour)	
	before injection	25—30 min after injection	before injection	0—60 min after injection
7	8.78±5.38	98.5±2.49	32.57±25.31	0

lysis of the content of epileptic activity replicated those obtained during the analysis of the numbers of bursts.

b) **Effect of CVP on hippocampal theta rhythm and cortical HVIA.** Table 1 shows the comparison of the content of theta rhythm and HVIA in the hippocampal and cortical records before and after injections of CVP (session II). In general, in the majority of rats of all groups, the content of theta rhythm before and after the injection was rather small and showed no consistent changes toward a decrease or an increase during the 3 hour recording period following the injection of CVP. On the other hand, the content of cortical HVIA tended to increase gradually, being at its peak in the last two analysed samples (120—125 min and 175—180 min after the injection). However, the analyses (parametric and nonparametric (Friedman)) ANOVAS revealed significant differences between groups as well as within groups neither in the theta nor in the HVS contents.

c) **Effects of physostigmine on hippocampal and cortical EEG.** As expected on the basis of literature data (6), in all the animals tested, injection of physostigmine resulted in a reduction of motor activity accompanied by an increase of theta content up to 90—100%. This effect reached its peak within 5—10 min and remained unchanged for 40—50 min. Cortical records contained no HVSE after physostigmine, and bursts of seizure activity were totally absent (Table 2).

d) **ChE determinations.** Determination of ChE revealed that 30 min after injections, in animals given physostigmine in dose of 1.0 mg/kg, enzyme activity decreased by 37% in the cerebellum, by 43% in the diencephalon, by 44% in the hippocampus, by 46% in the brain stem and by 52% in the frontal pole. The corresponding values in plasma and erythrocytes were 55.4% and 47.5%, respectively.

DISCUSSION

The main facts discovered in the present studies are:

1. The lack of a significant, either acute or long-lasting effect of

CVP intoxication on spontaneous seizure activity in the rat cortex, and the rather negligible reduction in the effectiveness of cardiazol when given after CVP. This is particularly striking when compared with the very strong inhibitory effect of the classic ChE inhibitor, physostigmine.

2. The contradictory effects of CVP and physostigmine on the theta content in the hippocampal EEG.

In our previous studies, in rats given CVP in a dose of 3.0 mg/kg, changes in general behavior (a decreased motor activity) were seen clearly up to 3 days after the exposure. A changed response to the introduction of a new object into the open field could be detected 10—14 days after the exposure in rats dosed with 3.0 mg/kg, as well as with 1.0 mg/kg of CVP (28). It appears from the present studies that the behavioral disruptions produced by CVP are not accompanied by clear-cut changes in electrocortical excitability or in hippocampal and cortical EEG or, that the changes, if they exist, could not be detected with the methods used.

There is no doubt that CVP is a very efficient ChE inhibitor, especially in the rat (7, 18). According to the data obtained earlier (28), 3 hours after 3.0 mg/kg dose of CVP, the activity of this enzyme in erythrocytes, in plasma and in different parts of the brain, is in all these cases about 10—15% of the control values. The recovery takes about two weeks in blood and in the brain alike. After the 1.0 mg/kg dose, ChE activity drops to about 50% after 3 hours and returns to a normal level within four days. On the basis of these data one might expect a rather strong and long-lasting activation of the central cholinergic system (due to an excess of free acetylcholine), which would be reflected by electrocortical arousal (desynchronized, low amplitude activity and no HVIA) and increased content of theta rhythm (immobility related theta or Type II theta) in the hippocampal record (6, 30). Cortical and hippocampal excitability are diminished during electrocortical arousal and the hippocampal theta, which is made apparent by the disruptive influence of arousing stimuli on ongoing seizure activity (16) as well as by a decrease in amplitude of cortical (16, 22, 29, 33) and hippocampal (35) evoked potentials during the theta rhythm. In the present studies, electrocortical arousal, the increased content of the theta rhythm and hippocampal excitability are diminished during electrocortical arous- pronounced after the injection of physostigmine but not after the injection of CVP in any of the doses tested. It is worth emphasizing here that in the case of physostigmine an almost continuous theta rhythm was present 25—30 min after the injection, i.e. when the supposed level of ChE activity in the brain was by about 50% lower than in the normal state.

One may argue that the stronger influence of physostigmine might be due to some changes in the cholinergic system produced by an earlier exposure to CVP. This, however, seems to be rather unlikely; the normalization of ChE after CVP takes no more than 14 days, and the effect of physostigmine in our experiments was quantitatively very similar to that observed by others after a similar dose (16). This suggests that the functional state of the brain cholinergic system of our rats was normal.

An increased content of the Type II theta after a single i.p. exposure to CVP was observed in our previous experiments on rabbits. However, it could be seen clearly only when the dose of CVP was sufficient to inhibit blood (plasma and erythrocyte) ChE activity well below 50% of the control value (17). Such a result was rather surprising considering the high responsiveness of the Type II theta generating system in this species; in a normal, conscious rabbit, as opposed to the rat, theta may be elicited by even slight changes in the environment (25).

Thus, the question arises, why even deep inhibition of the brain and blood ChE by CVP, but not by physostigmine, is so ineffective in the activation of the Type II theta generating system? It is known that, apart from inhibiting ChE, some OP-s as well as some carbamates, may exert agonist, or antagonist-like (1, 13) action on cholinergic postsynaptic receptors. Accordingly, the effect on receptors may enhance or negatively influence that resulting from ChE inhibition (i.e. from the excess of free acetylcholine). From the above, it may be assumed that the differences between the effects of physostigmine and CVP on the hippocampal and cortical EEG-s were due to differences in the ability of these compounds to interact with cholinergic receptors and to differences in the functional outcome of this interaction. The antagonist-like action of CVP on cholinergic receptors seems to be the best explanation of the results obtained in the present studies, although the agonist-like action of physostigmine cannot be excluded (1). However, the high acute toxicity of CVP, especially for rodents (18), suggests that such action might concern muscarinic receptors only, or even simply a part of these receptors.

Summing up, the obtained results show that i) the statement that brain electrical activity is the most sensitive index of OP-exposure (9, 10) does not hold true in the case of CVP, and ii) similar levels of ChE inhibition by different compounds may be accompanied by quantitatively different changes in brain electrical activity. Consequently, the level of ChE inhibition is predictive neither for the neurotoxicity of a given OP nor for the severity of other symptoms. It is difficult to tell at present whether the assumed antagonist-like action of CVP on cholinergic re-

ceptors might be regarded as beneficial for the exposed subject. Certainly, it might prevent some symptoms from appearing or at least reduce their severity. However, as our previous data suggest, the lack of acute symptoms of CVP exposure is not indicative of the appearance of subtle behavioral disturbances which may be detected even after the recovery of ChE (28).

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