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TOLERANCE TO CHLORPHENVINPHOS IN RATS ASSESSED ON THE BASIS OF CHANGES IN LOCOMOTOR BEHAVIOR IN ROTATING WHEELS

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Abstract. Spontaneous locomotor activity in rotating wheels was investigated in rats exposed repeatedly (i.p. daily injections, five days a week for two weeks) to an agricultural organophosphorus pesticide, chlorphenvinphos (CVP) at doses of 1.0 and 3.0 mg/kg. After a seven day interval each rat was injected with a single 3.0 mg/kg test dose of CVP in order to assess the stability of tolerance. Concomitant changes in blood and the brain ChE activity were also investigated. It was found that exposure to CVP at a low dose (1.0 mg/kg), resulting in less than 50% reduction of ChE activity in blood and in the brain, did not produce changes in spontaneous locomotion in rotating wheels in the rat. Higher doses (3.0 mg/kg) inhibited blood and the brain ChE by more than 50% and reduced locomotion. Under conditions of repeated exposure to CVP at the symptomatic (3.0 mg/kg) dose ChE activity remained low throughout the exposure period, however, locomotor activity returned to a normal level, i.e. tolerance developed, within less than five days. Seven days after termination of the repeated exposure, the behavioral subsensitivity to CVP still remained. The biochemical data suggest that it may be related, at least partially, to a diminished vulnerability of ChE in some parts of the brain to CVP induced inhibition.

INTRODUCTION

The repeated exposure to organophosphorous compounds (OP), irreversible inhibitors of cholinesterase (ChE), often results in development of tolerance, i.e. disappearance of the subject sensitivity to the compound under study. This effect manifests itself in a progressive diminution and, eventually, in a lack of acute vegetative and behavioral symptoms of intoxication after successive daily doses of a given OP.

Development of tolerance reduces the risk of death in the case of acute, heavy exposure (2, 16). In the persons exposed, however, it may lead to negligence of the

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danger. Such an attitude may create a serious risk, especially for agricultural workers during the spraying season. It may result in prolongation of the exposure period or increased probability of further, incidental exposure and, in consequence, in the development of more insidious effects, not necessarily connected with the cholinergic system (3). Moreover, recent studies on animals show that the development of tolerance depends on the kind of OP; i.e. the subject may become tolerant to one particular OP but not to another (12, 20) Therefore, testing the tolerance seems to be an important part of the complex studies on the toxicity of a given OP.

This study has focussed on chlorphenvinphos (Phosphoric acid, 2-chloro-1/2, 4-dichlorophenyl/vinyl diethyl ester – CVP), an OP pesticide, widely used in Poland. Some previous studies were aimed at finding out the behavioral and electrophysiological effects of single (5.6) and repeated (4, 7) i.p. exposure to this compound in the rat and rabbit, with particular regard to the effects detectable after normalization of ChE activity in blood and the brain. The purpose of the present experiments was to find answers to the following questions: 1) what is the rate of development of tolerance to CVP in the course of repetitive exposure? 2) what is the relationship between the tolerance development and changes in blood and the brain ChE?

Spontaneous locomotor activity in a rotating wheel served for the assessment of tolerance to CVP. This test has been selected for the following reasons: 1) running in rotating wheels is a complex behavior requiring integrity of the sensorimotor and motivational systems and, as such, it is very sensitive to changes in animal health status; 2) reduction in spontaneous locomotor activity is one of the most evident signs of acute OP intoxication.

MATERIALS AND METHODS

Animals

The experiment was performed on male Wistar rats from IMP-DaK stock (outbreeds), 350–400 g of body weight and 12–15 weeks old; 52 animals were used for the biochemical part of the experiment (ChE determinations), 15 served for the behavioral studies. The rats were housed in cages (55 × 35 × 20 cm) (four animals per cage in the case of rats used for ChE determinations and one per cage in the case of those used for behavioral study), and kept under standard laboratory conditions (temperature: 22–23°C, humidity 55–58%, light/dark cycle: 12/12 h with a light on at 6 a.m. and off at 6 p.m.).

Apparatus and recording

The home cages of the rats used for behavioral studies were equipped with rotating wheels. The circumference of the wheel was 70 cm and the width of the runway was 10 cm. The runway was made of a brass, tin covered net with the width of the net eyes of 0.6 cm. The wheels were mounted inside the cages, in the close vicinity of the back wall, in such a way that the lowest point was 7 cm above the floor level. The animal could go in or outside the wheel freely through a large opening. Each turn of the wheel by 90 degrees generated one square electrical pulse.

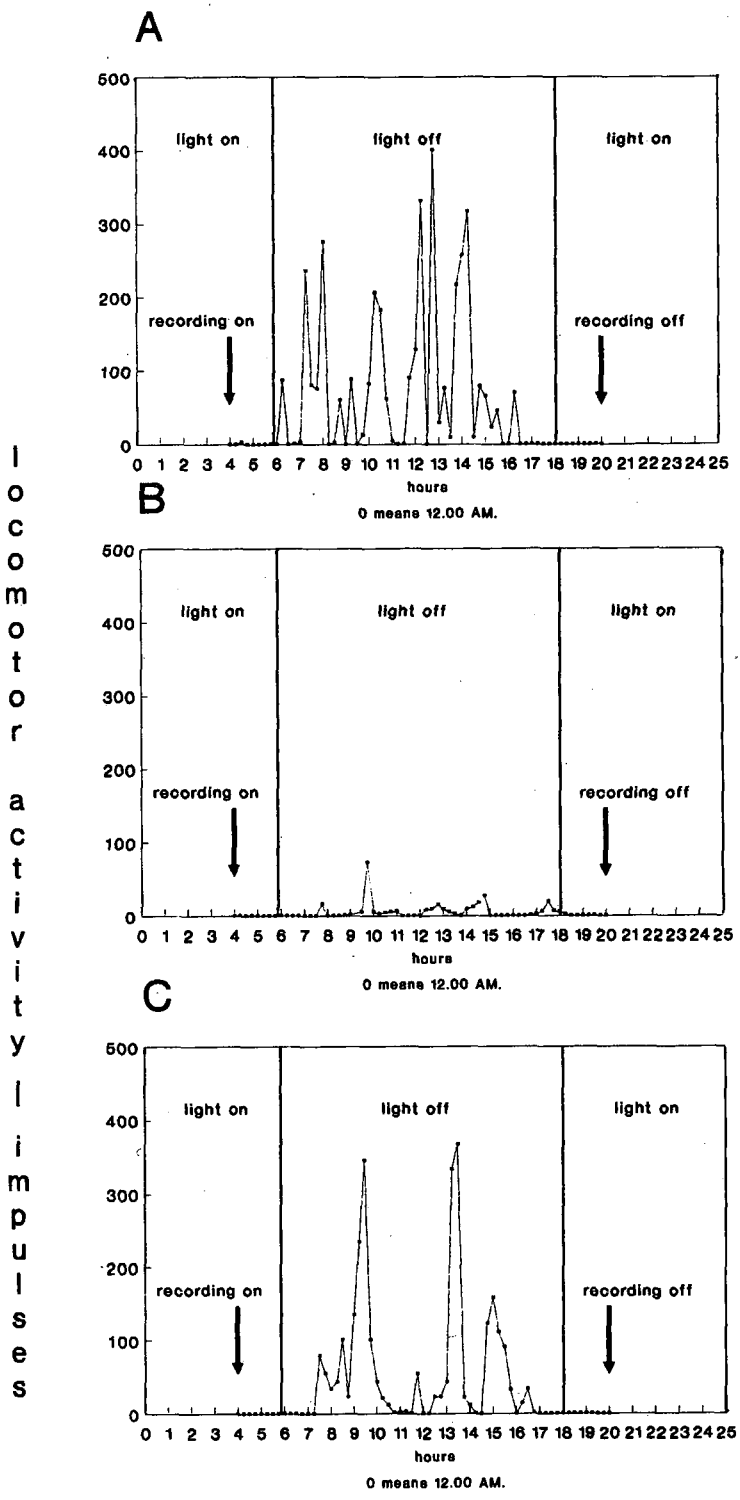


Fig. 1. Three consecutive individual records illustrating temporal distribution of locomotor activity on the day before exposure (A), during the first day of exposure (B) and fifth day of the exposure (C) to 3 mg/kg CVP.

The pulses were recorded in the mass memory of an Apple II Euro + microcomputer and grouped into 15 min bins. The recording started at 4 p.m. and ended at 8 a.m. of the next day.

The pesticide and the exposure conditions

CVP, technical grade, was obtained from the manufacturer (Organika-Azoty, Jaworzno, Poland). Before administration it was diluted in sterile, warm olive oil to the required concentration. The injections were made intraperitoneally. The volume of the injected solution was 0.5–0.7 ml.

Behavioral procedure

Prior to experiment the animals remained undisturbed in their home cages for two weeks (pre-exposure period). During the next two weeks (exposure period) each animal was injected every day, except for Saturdays and Sundays, with the prescribed dose of CVP in oil or oil alone. Five rats obtained CVP at a daily dose of 1.0 mg/kg (Group L), five at a dose of 3.0 mg/kg (Group H), and five were injected with oil alone (Group O). The injections were performed at 3–4 p.m. Then six days with no injection followed. On the seventh day all rats (including Group O) were given 3.0 mg/kg CVP i.p. (test dose). After another three days the experiment was terminated. Activity of each animal in its rotating wheel was recorded during the whole experiment. An example of the daily record in a graphical form is presented in Fig. 1. Statistical analysis was made using data obtained at the periods shown in Fig. 2.

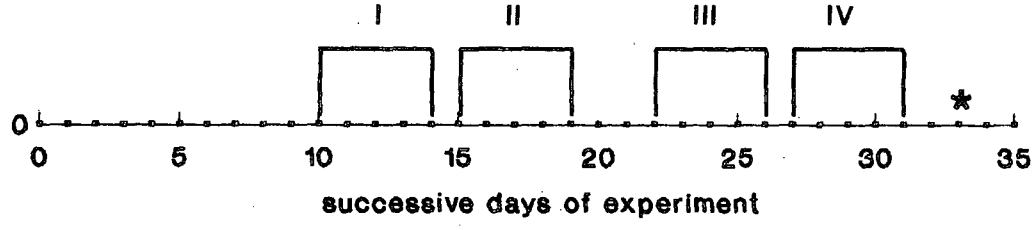


Fig. 2. Scheme of the course of experiment:
I – first period – 5 days before exposure
II – second period – first 5 days of exposure to CVP
III – third period – second 5 days of exposure to CVP
IV – fourth period – 5 days after exposure to CVP
* – test injection of CVP (3 mg/kg i.p.).

Biochemical procedure

The animals assigned for biochemical studies were given CVP in the same way and the same order as those in behavioral studies. The doses of the pesticide were 1.0 mg/kg (Group L, n = 24) and 3.0 mg/kg (Group H, n = 24). Some animals were given oil alone (Group O, n = 4). ChE activity in blood and selected parts of the brain was determined spectrophotometrically after Voss and Sachsee (18) at the

following time points: 3 and 24 hrs after the first injection, 3 hrs after the fifth injection, 3 hrs after the tenth injection, 7 days after the tenth injection and 3 hrs after the last (3.0 mg/kg) injection. For each ChE determination, four to five animals from each group were killed by decapitation. Details of the procedure have been described in our previous work (17).

Statistical analysis

The two way ANOVA and Tukey test were used for statistical evaluation of the behavioral as well as biochemical data.

RESULTS

Body weight

Changes in body weight of the animals during the experiment are presented in Fig. 3. No significant differences between the groups were found.

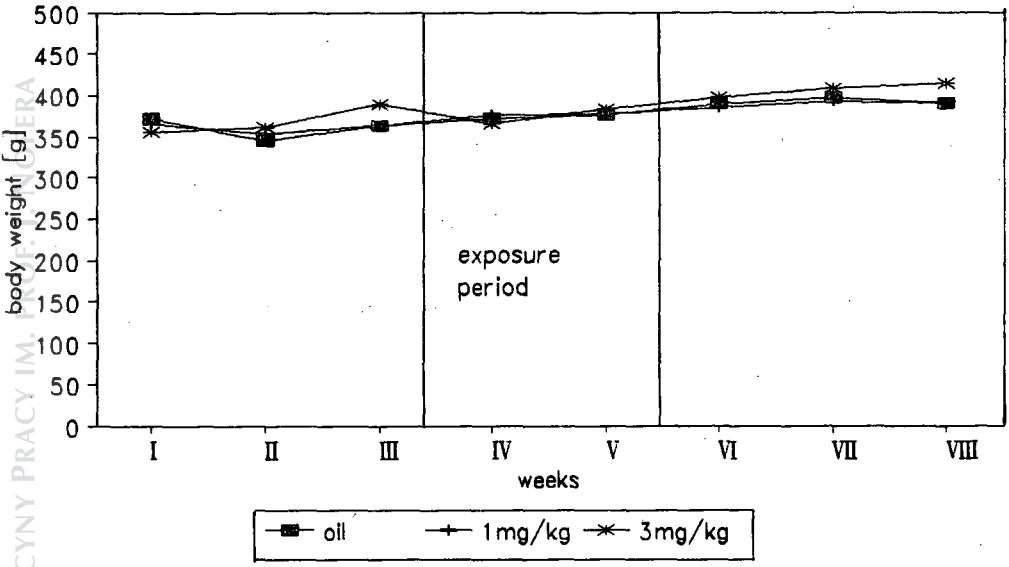


Fig. 3. Effect of i.p. exposure to CVP on body weight in the rats.

Behavioral data

The daily motor activity in the rotating wheels increased gradually attaining a plateau by the end of the pre-exposure period. The animals were active only in dark hours. Similarly during the exposure period, the activity was limited to dark hours. No clear-cut changes in the temporal distribution of counts were noted (Fig. 1). There were quite large differences between animals in the distance "traveled". In order to assess the changes resulting from the exposure the activity during the

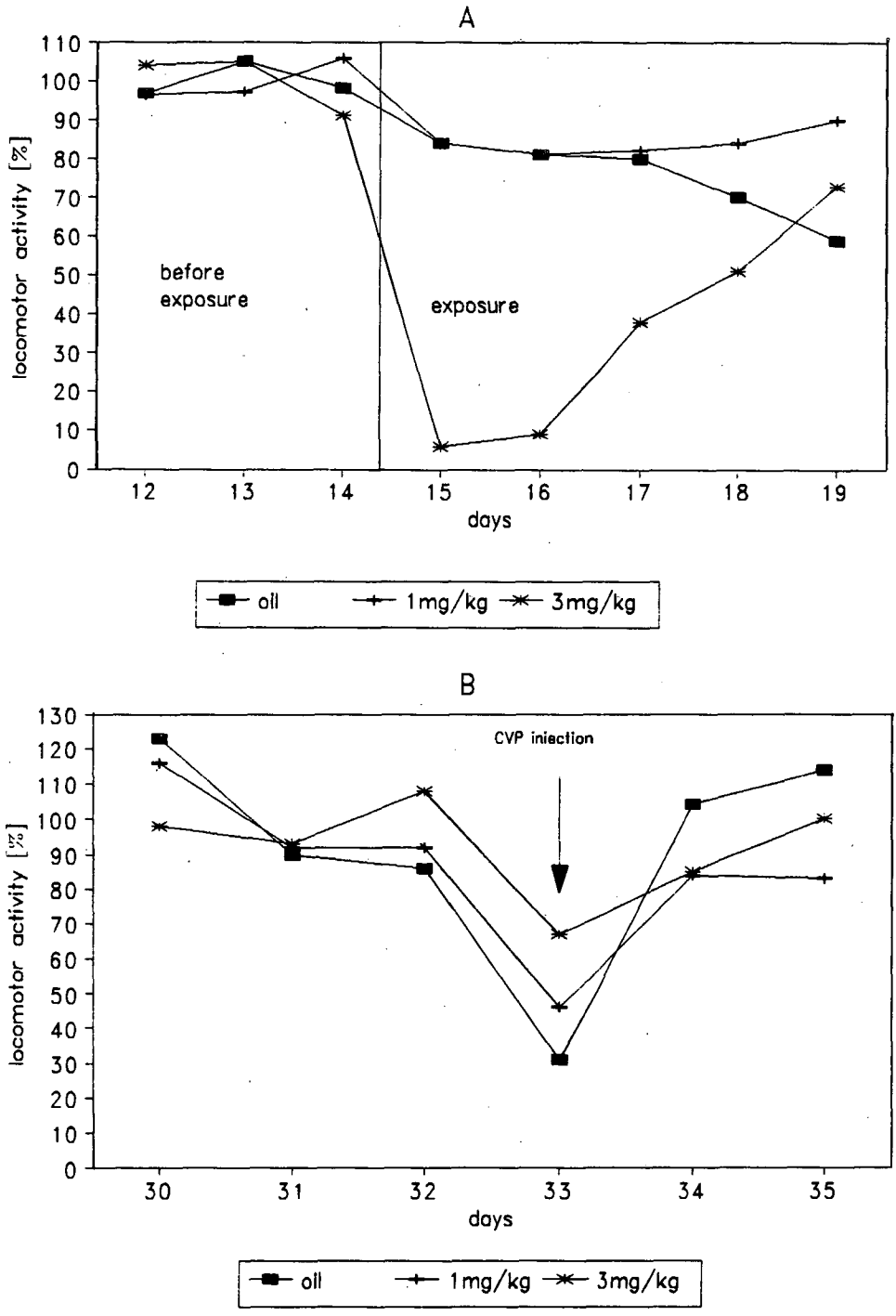


Fig. 4. Effects of exposure to CVP on locomotor activity in rotating wheels. A – last three days of pre-exposure period (I) and first five days of exposure period (II). B – effect of single injection of 3 mg/kg on the seventh day after discontinuation of the repeated exposure.

exposure period in each animal was expressed as a percent of the mean activity (i.e. mean numbers of counts) during the last three days of the pre-exposure period.

Changes in the total level of activity (a decrease) were seen only during the first five days of exposure (period I, Fig. 4A). The ANOVA (groups × days) revealed a significant group effect ($F2, 12 = 5.85$, $p < 0.05$) and significant group × days interaction ($F2, 12 = 13, 14$, $p < 0.001$). Detailed comparisons revealed that only in group H, and only on days 1, 2, 3 and 4 of the exposure period was the activity significantly lower than during the pre-exposure period (Fig. 4A).

Injection of 3 mg/kg of CVP to all animals after the six day period with no exposure resulted again in a decrease in activity (Fig. 4B). This effect, however, was not the same in all groups (effect of days: $F1, 12 = 13.09$, $p < 0.005$, interaction: $F2, 12 = 6.82$, $p < 0.05$). A comparison between successive days (three days before the injection as reference and three days following the injection) revealed significant differences only in group O ($F5, 60 = 9.95$, $p < 0.0001$) and in group L ($F5, 60 = 4.68$, $p > 0.005$). In both groups only on the day directly following the injection was the activity significantly depressed. In group H the changes were not significant. Additional comparisons revealed that in group O as well as in group L the reduction in activity produced by the test injection was significantly smaller than that seen in group H after the first injection of CVP at the same dose ($p < 0.01$ in both cases).

Biochemical data

In rats sacrificed three hours after the first injection the level of ChE activity in plasma and erythrocytes was lowered by 35–45% in the case of the L group and 88–92% in the case of the H group. The level of ChE inhibition in the selected parts of the brain was similar and approached that in blood. In the case of erythrocytes and brain structures the ChE determinations made 3 hrs after the fifth and the tenth injections suggested no further significant changes, decrease or increase, during the exposure in both exposed groups. The plasma ChE, however, showed an obvious tendency toward normalization in the course of the exposure, at least in the H group.

By the end of the six day period after the repeated exposure, the plasma ChE activity was above normal in group L and normal in group H. The erythrocyte ChE activity, on the contrary, was still below normal level in both groups; mean 67% and 49% in group L and H, respectively (Fig. 5). In the brains of group L (Fig. 6A) the ChE activity increased above 80% in all parts studied. In group H (Fig. 6B) the rate of ChE normalization was apparently different in different parts of the brain; it was the fastest in the case of the cerebellum and the slowest in the case of the hippocampus.

In group L and H 3 hrs after the injection of the test dose of CVP (3 mg/kg) the ChE activity in plasma and in erythrocytes decreased to a level nearly the same or slightly above that noted 3 hrs after the tenth injection (Fig. 5). As regards the brain, in group L the test injection resulted in a marked decrease in ChE activity in all parts; the effect being stronger in the case of the diencephalon and weaker in the case of the cerebellum. Generally, in all parts the level of ChE activity after the test injection approached that found after the first injection of CVP with the 1.0 mg/kg dose. In group H, the test injection resulted in a significant decrease in ChE activity only in the case of the cerebellum and anterior part of the hemisphere (Fig. 6B). It is to be noted that, 3 hrs after the test injection, the relative ChE activity in particular parts of the brain in group H and L did not differ ($p > 0.05$ in all cases).

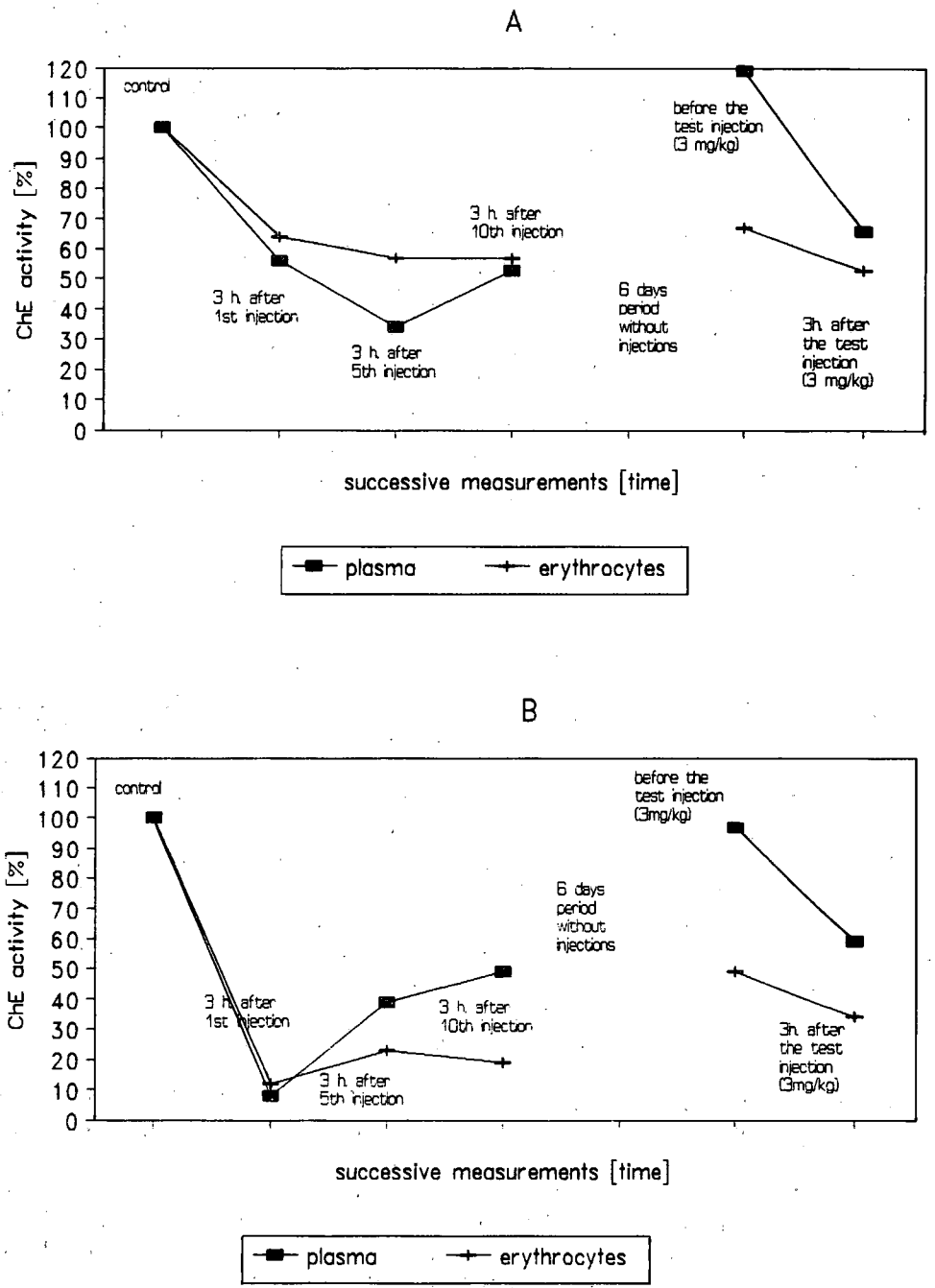
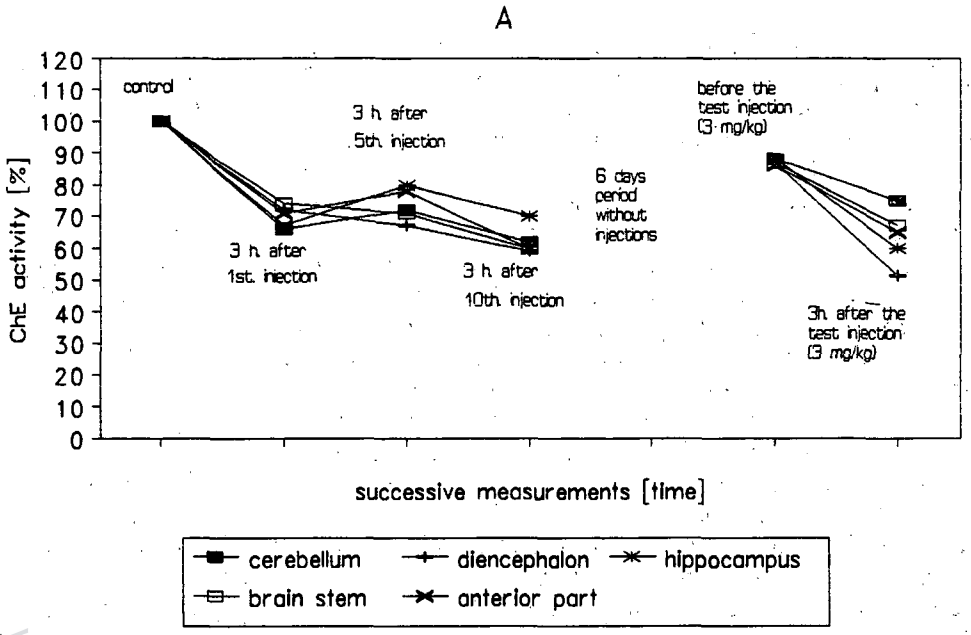


Fig. 5. ChE activity in blood of rats exposed repeatedly to CVP in dose of: A — 1 mg/kg and B — 3 mg/kg body. weight.



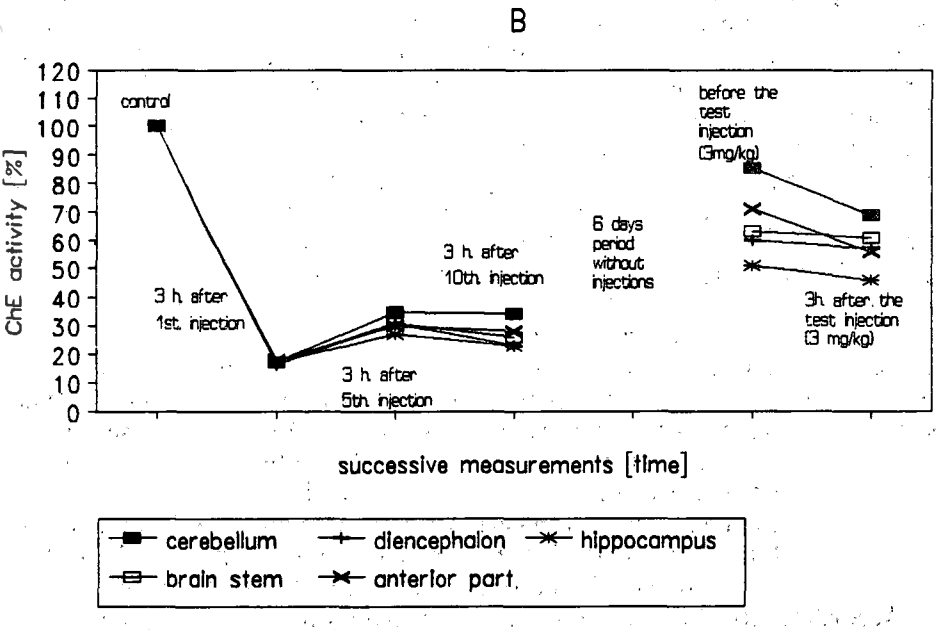


Fig. 6. ChE activity in different parts of the brain in rats exposed to CVP in dose of: A — 1 mg/kg and B — 3 mg/kg body weight.

DISCUSSION

The studies on humans suggest that subjective symptoms of OP intoxication usually appear when the erytrocyte ChE falls below 50% of the pre-exposure level (19). In most behavioral studies performed on animals the selected, effective doses produced more than 50% ChE inhibition in blood and in the brain (e.g. 9,17). In the light of these data, it is not surprising that in our present experiments, a decrease in locomotor behavior in rotating wheels occurred only in the H group. In the course of repeated (daily) exposure, the effectiveness of CVP in producing depression of locomotor activity in this group disappeared, i.e. tolerance developed, within less than five days. A similar rate of normalization of spontaneous locomotor activity was found in the case of repeated exposure to DFP (15) and soman (14). It has been found however, that in the course of repeated OP exposure, different funtions may show different rates of normalization; vegetative functions seem to normalize sooner than the motor ones. Some effects may persist during the whole course of the exposure (15). The data obtained in our present experiments do not allow us to draw conclusions concerning CVP effects on separate physiological functions. As has already been mentioned in the introduction, locomotin in rotating wheels is a complex form of behavior requiring integrity of motor, sensory and motivational systems. The disappearance of differences in the number of counts within less than five days of the repeated esposure to CVP suggests that this time was sufficient for adaption of the basic functions to the changed internal conditions, i.e. profoundly lowered ChE activity.

The effects of the test dose of CVP applied after the seven day interval are interesting for two reasons. Firstly, they show that the acquired tolerance to high doses of this pesticide lasts longer than one week, and that repeated exposure to lower doses does not produce tolerance to high doses. Secondly, it appears that the maintenance of behavioral subsensitivity to CVP may be related to biochemical tolerance which is suggested by the lack of changes in ChE activity in some parts of the brain in group H after the test dose. It should also be noted that the behavioral effect, i.e. decrease in locomotor activity, observed in group O,3 hrs after injection of the test dose of CVP, was markedly smaller than that in group H after the first injection of the pesticide. This indicates that the O group was less sensitive to CVP than could be expected. One of the factors which may be responsible for this difference is a reduction of the stress associated with the injection procedure. Another may be the three week access to rotating wheels, i.e. physical training. The lack of significant decrease in rotating wheel activity after the beginning of the repeated exposure in the O group allows one to reject the first possibility. There are some data, however, which suggest that prolonged physical exercise may lead to a marked diminution of suppressive effects of ChE inhibitors on behavior (11). The lack of significant effect of the test dose on ChE activity in some brain regions in group H, and the literature data mentioned above, allows one to assume, that the tolerance noted in this group during the test is a combined effect of physical exercise and some adaptive changes induced by the repeated CVP exposure.

According to the literature several mechanisms may be involved in the development of tolerance to OPs. Well documented is the reduction in density of postsynaptic, muscarinic receptors (1, 8, 10, 13, 15). The development of this adaptive

change takes about four days and is maintained as long as decreased ChE activity persists (15). It corresponds well with the return of the locomotor activity in group H in our experiments. Another possibility is a change in the proportion of ChE isoenzymes in such a way that some, which are less vulnerable to the given OP, recover faster and contribute predominantly to the total ChE activity (14). The lack of (hippocampus, brain stem and the anterior part of the hemisphere) or small (blood, diencephalon, cerebellum) changes in ChE activity 3 hrs after the test dose in group H, when compared with the effects of the first injection, suggest that in the case of CVP also the latter mechanism is involved in the development and maintenance of behavioral subsensitivity.

Surprisingly, in groups L and H, three hours after the test injection (3.0 mg/kg), the levels of ChE activity in the brain were similar. The decrease in locomotor activity, however, occurred only in group L. In addition, in group L, the levels of ChE activity in the brain structures were not lower after the test injection than those found after the first one (1.0 mg/kg), beginning the repeated exposure. The behavioral change, however, was evident only after the test dose. It may suggest that the behavioral effectiveness of CVP is related not only to its action on ChE.

To sum up, the results of the present experiments have shown that in the course of repeated exposure to CVP at symptomatic doses, the effectiveness of the compound in producing changes in complex locomotor behavior diminishes gradually at a rate similar to that found in the case of some other OPs. Acquired behavioral subsensitivity persists longer than seven days after termination of the exposure. The biochemical data suggest that it may be related, at least partially, to a diminished sensitivity of ChE in some parts of the brain to CVP-induced inhibition.

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