

AMPHETAMINE- AND SCOPOLAMINE-INDUCED LOCOMOTOR ACTIVITY FOLLOWING TREATMENT WITH CHLORPHENVINPHOS OR CHLORPHYRIPHOS IN RATS

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Abstract

Objectives: Effects of acute exposure to organophosphorous pesticides (OPs), chlorphenvinphos (CVP) or chlorphyriphos (CPF) on amphetamine (AMPH)- or scopolamine (SCOP)-induced open-field locomotion were compared in rats. **Materials and Methods:** CVP and CPF were administered intraperitoneally, both at doses resulting in about 50% inhibition of erythrocyte acetylcholinesterase (rbcAChE). The pesticide groups did not differ one from another in the magnitude of the acute behavioral effects. **Results:** Three weeks after the exposure, i.e. when AChE activity returned to normal level, the behavioral response to AMPH and SCOP was significantly reduced in CVP-, but not in CPF-pretreated rats. **Conclusions:** These results confirm that a single exposure to organophosphorous pesticides may result in neurobehavioral effects detectable after restitution of AChE. They also show that CVP and CPF differ in respect of long lasting functional consequences of exposure, which suggests a difference in the mechanism of toxicity.

Key words:

Organophosphorous pesticides, Amphetamine, Scopolamine, Rat

INTRODUCTION

Organophosphates form a large group of chemical substances with numerous applications. Many of organophosphates are active components of commonly used pesticide preparations and may contaminate soil and crops [1]. The biocidal activities of organophosphate pesticides (OPs) derive from their potential for irreversible inhibition of acetylcholinesterase (AChE). This property is common to all OPs, but particular compounds differ considerably in the level of acute toxicity [2]. The acute toxic effect of OPs is manifested by a number of subjective and objec-

tive symptoms directly related to increased acetylcholine (ACh) availability and subsequent overstimulation of cholinergic receptors (both muscarinic and nicotinic) in the brain and peripheral organs [3]. Apart from acute effects, OPs exposure may lead to adverse neurotoxic effects that may be detected long after full restitution of AChE activity. The best known is the organophosphate-induced delayed neuropathy (OPIDN), which results from the damaged axonal transport [4,5]. Along with OPIDN, alterations in the central nervous function may also occur. The clinical studies and epidemiological data point

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to long-lasting disturbances in the central nervous system (CNS) functions in person with a history of OP exposure. The results indicate an impairment of cognitive functions (memory failure and concentration deficit) and emotional disturbances (depression, excitability, irritability) [6–10]. Long-lasting changes in CNS functions following OP exposure were also found in laboratory animals [11,12]. The neuronal basis of these effects has not yet been elucidated. However, certain observations suggest that at least some of them may be related to an elevated sensitivity of the CNS cholinergic system [13]. Results from our earlier experiments support this suggestion. We found that rats pretreated with chlorfenvinphos ((2-chloro-1(2,4-dichlorophenyl) vinyl diethylphosphate – CVP)) at a single dose of 1.0 mg/kg (1/10LD₅₀) and tested after full recovery of AChE activity, shared some characteristics with rats showing inborn cholinergic hypersensitivity [14], namely low behavioral sensitivity to dopaminergic (DA) agonists and muscarinic antagonists [15,16]. It appears, however, that the effects produced by other OPs may be qualitatively different. In the experiment of Pope et al. [17], rats subcutaneously exposed to chlorpyrifos (diethyl 3,5,6-trichloro-2-pyridine thiophosphate – CPF) showed an augmented behavioral response to scopolamine (SCOP) challenge weeks after the exposure. The contrasting effects of CVP and CPF on the sensitivity to SCOP may support the conjecture that long-lasting consequences of OP exposure are determined by some specific properties of the given OP unrelated to its ability to inhibit AChE [12]. It cannot be excluded, however, that the differences between our results and those reported by Pope et al. [17] are attributable rather to differences in experimental procedures than to the use of different pesticides. In our experiment, the rats were challenged with SCOP only once, 3 weeks after exposure, while rats in the aforesaid experiment [17] were given SCOP repeatedly at two-week intervals starting from the day of exposure. This means that behavioral response to a subsequent SCOP challenge dose could be affected not only by CPF pretreatment, but also by prior SCOP injections. The purpose of the experiment reported here was to compare long-lasting changes in the intensity of behavioral responsiveness (open-field locomotion) to amphet-

amine (AMPH) and SCOP in rats treated with CVP or CPF at doses equipotent to AChE inhibition. Quantitative and qualitative similarities of the CVP and CPF treatment effects may indicate that both result from past AChE inhibition. Lack of similarity might indicate that properties other than the ability to inhibit AChE should also be taken into account as possible determinants of long-lasting consequences of OP exposure.

MATERIALS AND METHODS

Animals

The experiment was performed on 114 male, outbred Wistar rats. The rats were 3–4 months old and weighed 320–350 g at the experiment onset. For two weeks before the start and during the experiment, they were housed in single rat cages at 22°C ± 0.5°C, with a light/dark cycle of 12/12 h (light on at 6 AM). Standard rat food pellets (Murigran) and tap water were accessible *ad libitum*. Body weight was measured routinely once a week and before each chemical administration. All experimental procedures, including animal handling and care, were approved by the local Ethical Commission and were carried out by suitably qualified personnel.

Chemicals and doses

The following compounds were used: CVP (technical grade) and CPF (technical grade) both obtained from ORGANIKA-AZOT Jaworzno, Poland, d-amphetamine (d-amphetamine sulphate – SIGMA), and SCOP (scopolamine hydrobromide – SIGMA). CVP and CPF were diluted in corn oil, while both AMPH and SCOP were dissolved in physiological saline (natrium chloratum 0.9%, POLFA). All solutions were given intraperitoneally (i.p.) at 1.0 ml/kg volume. Both pesticides were administered only once at 1.0 mg/kg (CVP) and 70.0 mg/kg (CPF). The CVP dose was the same as that used in our earlier experiments [15,16]. The CPF dose was established in a pilot experiment and was equivalent to the CVP dose with respect to the level of erythrocyte acetylcholinesterase (rb-AChE) inhibition. Doses of AMPH and SCOP (1.0 and

0.75 mg/kg, respectively) were identical to those employed in our earlier experiments [16].

Biochemical analysis

In the biochemical part of the experiment, the activity of rbcAChE and plasma cholinesterase (ChE) were determined. This test (cholinesterases activity determinations) was performed to confirm the equivalence between the level of rbcAChE inhibition and pesticide doses and to determine the time of rbcAChE recovery. Determinations were performed on 18 rats divided into three groups: the control, CVP, and CPF groups ($n = 6$ in each group). The rbcAChE and plasma ChE activities were determined in peripheral blood drawn from the tail vein into heparinized glass capillary tubes. The blood samples were taken at the following time points: seven days before the exposure, at the time of maximum enzyme inhibition (3–4 h after the pesticide treatment), and then on posttreatment days (PTD) 7, 14 and 21. The enzyme activities were determined by Ellman's spectrophotometric method [18] as described in details in earlier reports from this laboratory [19,20]. In order to determine plasma ChE, 200 μ l samples of fresh blood were suspended in buffered DTNB solution (0.3 mM, pH 7.8, SIGMA) in 1/1000 proportion and carefully centrifuged at 3000 rpm for 5 min. The supernatants were used for further determination. The difference between the whole blood and plasma activities was regarded as rbcAChE activity. The enzyme activities were expressed in international units (UI).

Behavioral tests

Behavioral tests were used to assess the open field locomotion before and after a challenge dose of AMPH or SCOP in pesticide pretreated rats.

Apparatus. A computerized 4-unit set of activity chambers (PORFEX Ltd, Białystok, Poland) was used. The set was located in a room, 6 • 2 • 3 meters, neighboring the animal rooms. It was illuminated with a row of four white luminescence bulbs located at the ceiling. The ambient temperature and humidity inside the testing room were the same as in the animal rooms. Each activity chamber consisted of clear acrylic open field box (63 • 63 • 40 cm)

with 2 tiers of infrared motion sensors spaced 2.5 cm apart. The first and second tiers of sensors were located 4.0 cm and 15.0 cm, respectively, above the floor level. Each cage was equipped with a calculating system, which transformed the beam interruptions into the location of the animal within the cage five times per sec. Raw data were stored in the cage memory. After termination of the test session, the cage memory content was downloaded to computer memory and subjected to further analysis with the aid of a computer program. The end-result of the analysis was the total distance (the sum, in meters, of all horizontal shifts at the point representing the location of the rat's body) covered by the rat in arbitrarily adopted time intervals (blocks).

Procedure and data analysis. All measurements of the locomotor activity were conducted between 7AM and 2 PM. At the beginning of the experiment, all animals were habituated to the activity chambers 2 times for 30 min with a 7-day interval. Then the animals were classified into twelve groups: four control groups (receiving pure oil), four CVP- and four CPF-treated groups (Table 1). The inclusion criteria comprised body weight and spontaneous locomotor activity, both determined on the day of the second 30-min habituation session. Pesticides (or pure oil) were administered 2–3 days after the last habituation session. The rat behavior after AMPH or SCOP challenge was evaluated twice, on PTD7 (7 days after treatment) and PTD21 (21 days after treatment), or only once, on PTD21. Each injection was preceded by the 20-min measurement of locomotor activity in the test cage. After the injection of pesticides or oil, the animals were transferred back to their home cages for 3–4 h and thereafter they were relocated again to test cages for 20 min. The aim of this procedure was to estimate the pesticide effect on the open-field locomotion at the time of maximum AChE inhibition. In sessions designed to measure behavioral responsiveness to AMPH or SCOP, the animals were transferred to test cages immediately after the drug administration and left there for 80 min. To compare acute effects of CVP or CPF, the values of the 20-min measurement performed 3–4 h after pesticide injection were expressed as the percentage of the 20-min preinjection results and analyzed using the



non-parametric, one-way ANOVA (Kruskal-Wallis test). Successive posttreatment measurements of body weight and cholinesterase activities were also expressed as the percentage of respective pretreatment values. In order to assess the response to AMPH and SCOP, the 80-min postinjection measurement was divided into four 20 min sections (blocks), numbered successively as blocks 1, 2, 3 and 4. The locomotor activity (covered distance) in each block was expressed as the percentage of the 20 min pre-injection measurement. The effects of pesticide exposure on body weight, cholinesterase activity and the response to AMPH and SCOP challenges were assessed with a two-way ANOVA. When a significant interaction effect was found, it was followed by one-way ANOVA and Tukey's test for pairwise comparisons [21]. In case of non-homogeneity of covariance, an approximation procedure, which enables to avoid assumption about equal covariance, was applied. In this procedure, the degrees of freedom used to find the critical values are reduced, thereby making the test more conservative [22]. Differences were considered statistically significant when the probability of the null hypothesis was 5% or less. In order to find out the time course of the exposure-induced changes in behavioral responsiveness to AMPH measurements (corresponding blocks) performed

7 and 21 days before exposure, the results were compared with use of the Wilcoxon test [21].

RESULTS

Biochemical investigations

rbcAChE activity

Comparison of *rbcAChE* activity indicated significant group effect ($F(2,15) = 12.91$, $p < 0.001$), measurement

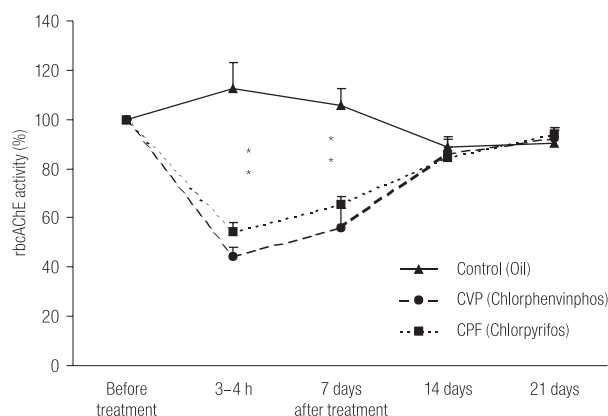


Fig. 1. Changes in *rbcAChE* activity in CVP- or CPF-treated rats. Unrelative pretreatment values of *rbcAChE* activity (UI) regarded as 100%: Control group – 0.7234 ± 0.119 , CVP group – 0.7184 ± 0.124 and CPF group – 0.7421 ± 0.131 ($n = 6$ in each group). ANOVA: group effect: $F(2,15) = 12.91$, $p < 0.001$, measurement effect: $F(1,15) = 16.75$, $p < 0.001$, interaction: $F(2,15) = 77.41$, $p < 0.001$. * $p < 0.001$ compared to the same measurements in the oil group.

Table 1. Groups used in the behavioral part and experimental protocol

Group	Number of animals	Treatment	Pharmacological challenge	
			TEST drug	Posttreatment day
O/A/7	8	Oil	AMPH (1.0 mg/kg)	PTD7
O/A/7/21	8	Oil	AMPH (1.0 mg/kg)	PTD7 and PTD21
O/S/7	8	Oil	SCOP (0.75 mg/kg)	PTD7
O/S/7/21	8	Oil	SCOP (0.75 mg/kg)	PTD7 and PTD21
CVP/A/7	8	CVP	AMPH (1.0 mg/kg)	PTD7
CVP/A/7/21	8	CVP	AMPH (1.0 mg/kg)	PTD7 and PTD21
CVP/S/7	8	CVP	SCOP (0.75 mg/kg)	PTD7
CVP/S/7/21	8	CVP	SCOP (0.75 mg/kg)	PTD7 and PTD21
CPF/A/7	8	CPF	AMPH (1.0 mg/kg)	PTD7
CPF/A/7/21	8	CPF	AMPH (1.0 mg/kg)	PTD7 and PTD21
CPF/S/7	8	CPF	SCOP (0.75 mg/kg)	PTD7
CPF/S/7/21	8	CPF	SCOP (0.75 mg/kg)	PTD7 and PTD21

O – Oil;

A – Amphetamine (AMPH – 1.0 mg/kg);

PTD7 – seven days after treatment (before *rbcAChE* normalization);

CVP – Chlorphenvinphos;

S – Scopolamine (SCOP – 0.75 mg/kg);

PTD21 – twenty one days after treatment (after *rbcAChE* normalization).

CPF – Chlorpyrifos;

PTD – posttreatment day;

effect ($F(1,15) = 16.75$, $p < 0.0001$) and groups • measurements interaction ($F(2,15) = 77.41$, $p < 0.0001$). Three to four hours after pesticide administration, rb-cAChE activity in the CVP and CPF-treated groups was about $54.2 \pm 10.6\%$ and $44.2 \pm 9.5\%$ of the pretreatment value and it was significantly lower in both groups respectively, than in the control group. The differences between the pesticide groups were insignificant. Similar differences between groups were found on PTD7, but there were no differences between groups on PTD14 and PTD21 (Fig. 1).

Plasma ChE activity

Comparison of plasma ChE activity revealed significant group effect ($F(2,15) = 21.42$, $p < 0.0001$), measurement effect ($F(1,15) = 156.53$, $p < 0.0001$) and groups • measurements interaction ($F(2,15) = 180.22$, $p < 0.0001$). Significant differences between the pesticide groups and the control group were found only in the measurements conducted between the 3rd and 4th h after treatment ($F(2,60) = 138.14$, $p < 0.0001$). At that time, plasma ChE activity was significantly lower in both pesticide groups compared to controls. However, in the

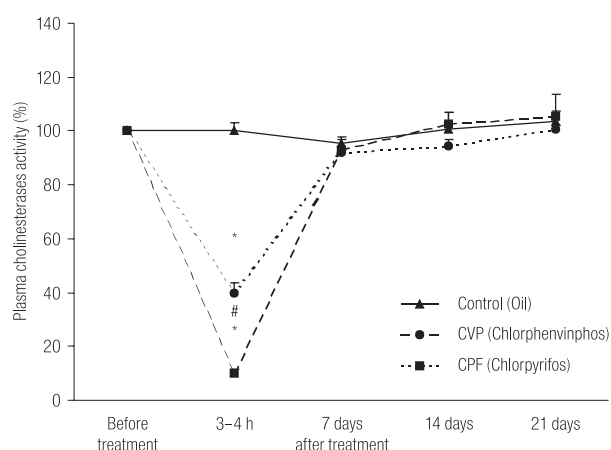


Fig. 2. Changes in plasma cholinesterases activity in CVP- or CPF-treated rats. Unrelative pretreatment values of plasma cholinesterases activity (UI) regarded as 100%: Control group – 0.2749 ± 0.091 , CVP group – 0.2843 ± 0.101 and CPF group – 0.2796 ± 0.089 ($n = 6$ in each group). ANOVA: group effect: $F(2,15) = 21.42$, $p < 0.001$, measurement effect: $F(1,15) = 156.53$, $p < 0.001$, interaction: $F(2,15) = 180.22$, $p < 0.001$. * $p < 0.001$ compared to the same measurements in the oil group; # $p < 0.001$ compared to differences between CVP- and CPF-treated groups.

CPF-treated group, ChE activity was suppressed more than in the CVP-treated group (to $10.01\% \pm 3.74\%$ and $39.56\% \pm 9.17\%$ of the preinjection value, respectively). No significant differences between groups were found in measurements conducted on PTD7, PTD14 and PTD21 (Fig. 2).

Effect of pesticide on body weight

To compare the effect of exposure on body weight, the data on rats used in the behavioral part of the experiment were analyzed. The analysis showed significant group effect: $F(2,45) = 38.45$, $p < 0.0001$, measurement effect: ($F(1,45) = 142.39$, $p < 0.0001$), and groups • measurements interaction ($F(2,45) = 4.38$, $p < 0.02$). In the control rats and CVP-treated groups, body weight increased gradually in the course of the experiment. The CPF-treated group showed a temporary decrease in body weight that continued for 7 days post exposure. After that time, the rats were gaining weight again at a rate similar to that in the control group, i.e. a compensatory increase was not observed. Thus, until the end of the experiment, body weight of the CPF rats was significantly lower compared to that of the control and CVP- treated rats (Fig. 3).

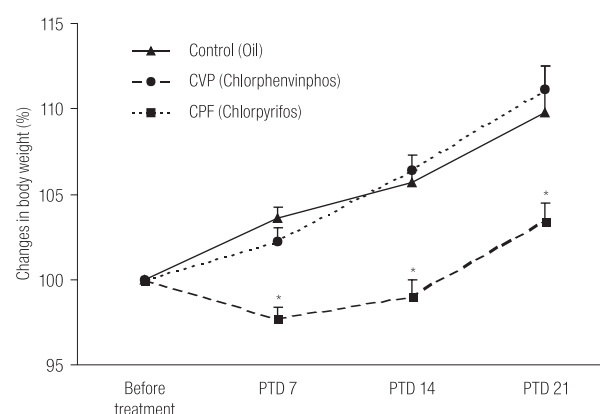


Fig. 3. Changes in body weight after treatment with CVP or CPF. Unrelative pretreatment values of body measurement (g) regarded as 100%: Control group – 340 ± 10 , CVP group – 330 ± 15 and CPF group – 335 ± 10 ($n = 16$ of each group). ANOVA: group effect: $F(2,45) = 38.45$, $p < 0.0001$, measurement effect: $F(1,45) = 142.39$, $p < 0.0001$, interaction: $F(2,45) = 4.38$, $p < 0.001$. * $p < 0.05$ compared to the control group.

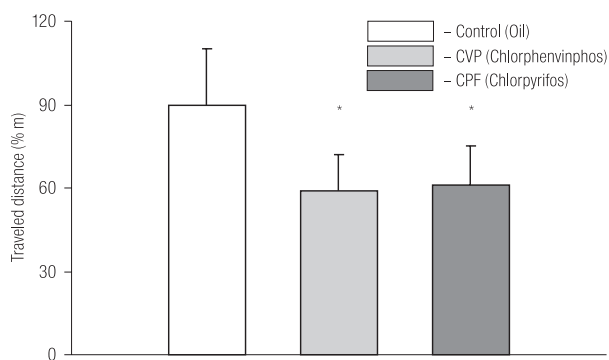


Fig. 4. Acute effects on locomotor activity in rats treated with CVP or CPF. Unrelative pretreatment values of traveled distance (m) during the 20-min measurement regarded as 100%: Control group – 47 ± 18 , CVP group – 53 ± 21 and CPF group – 51 ± 19 ($n = 32$ in each group). ANOVA (Kruskal-Wallis test): $X^2 = 13.752$, $df = 2$. * $p < 0.05$ compared to the control group.

Behavioral tests

Acute effects of CVP and CPF on the rat motor activity

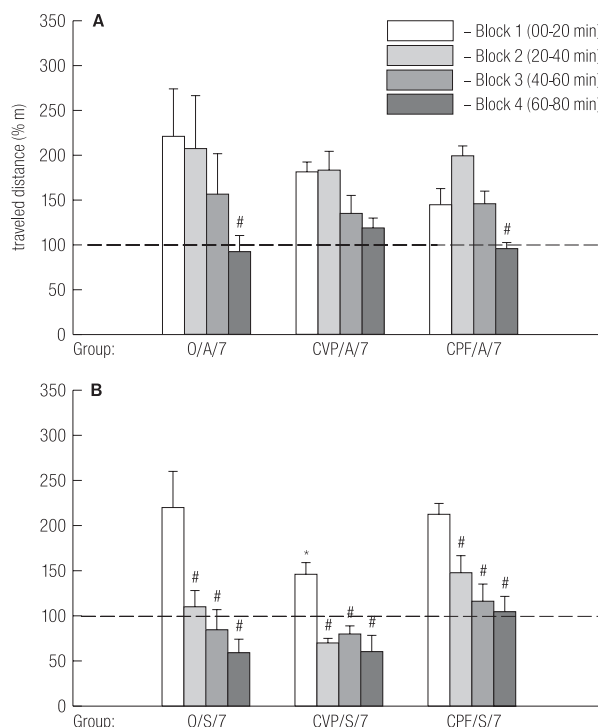
In the CVP and CPF groups, the locomotor activity, measured between the 3rd and 4th h after treatment, was significantly suppressed compared to the control group ($\chi^2 = 13.75$, $df = 2$, $p < 0.002$), but the pesticide groups did not differ one from another (Fig. 4). In none of the pesticide-treated groups symptoms characteristic of OP poisoning (salivation, diarrhoea, tremor, fasciculations) were observed.

Response to pharmacological challenge on PTD7

Amphetamine. In all groups, AMPH increased the locomotor activity, and the maximal effects were observed in blocks 1 and 2 (00–40 min after AMPH administration). A significant groups • blocks interaction $CF(2,21) = 8.31$, $p < 0.005$ was found. Differences between blocks were found in O/A/7/21 ($F(3,63) = 15.23$, $p < 0.0001$), and CPF/A/7/21 ($F(3,63) = 7.99$, $p < 0.005$): in both these groups, the locomotor activity in the last block was significantly lower than in the preceding blocks. However, no significant differences between groups were found in any of the blocks. The results suggest no significant alterations in behavioral sensitivity to AMPH on PTD7 following treatment with 1.0 mg/kg CVP or 70.0 mg/kg CPF.

Scopolamine. Compared to the AMPH effect, the locomotion stimulating effect of SCOP appeared more rapidly and the differences between groups were more appar-

ent (group effect: $F(2,21) = 4.57$, $p < 0.03$; block effect: $F(1,21) = 30.25$, $p < 0.0001$); group • block interaction: $F(2,21) = 5.23$, $p < 0.02$). Detailed comparisons showed significant differences between groups in block 1 ($F(2,84) = 4.38$, $p < 0.02$) and block 2 ($F(2,84) = 4.02$, $p < 0.05$). The CVP/S/7/21 group was the source of these differences. In this group, the locomotor activity in block 1 was significantly lower than in the O/S/7/21 and CPF/S/7/21 groups, while in block 2 it was significantly lower than in the CPF/S/7/21 group. The CPF/S/7/21 group did not differ from the corresponding oil group in any of the blocks. SCOP-evoked increase in locomotor activity was most evident in



AMPH – 1.0 mg/kg; SCOP – 0.75 mg/kg.

Fig. 5. Effects of AMPH and SCOP on the locomotor activity in rats treated with oil, CVP or CPF 7 days after pesticides treatment (before restitution of rbcAChE activity). **A.** Unrelative values (before AMPH challenge) of traveled distance (m) during the 20-min measurement regarded as 100%: O/A/7 – 32 ± 6 , CVP/A/7 – 35 ± 8 and CPF/A/7 – 31 ± 9 ($n = 8$ in each group). ANOVA: group effect: NS, block effect: $F(1,21) = 14.51$, $p < 0.05$, interaction: $F(2,21) = 8.31$, $p < 0.005$. # $p < 0.0001$ compared to block 1 in the same group. **B.** Unrelative values (before SCOP challenge) of traveled distance (m) during the 20-min measurement regarded as 100%: O/S/7 – 29 ± 8 , CVP/S/7 – 32 ± 7 and CPF/S/7 – 28 ± 11 ($n = 8$ in each group). ANOVA: group effect: $F(2,21) = 4.75$, $p < 0.03$, block effect: $F(1,21) = 30.25$, $p < 0.0001$, interaction: $F(2,21) = 5.23$, $p < 0.02$. * $p < 0.05$ compared to the same block in the O/S/7, # $p < 0.0001$ compared to block 1 in the same group.

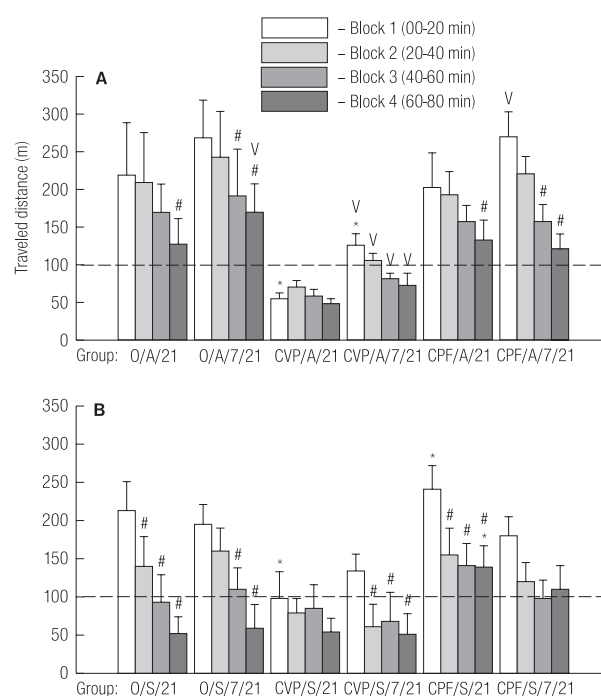
block 1 (in all groups, the locomotor activity in block 1 was significantly higher than in the remaining blocks) (Fig. 5). This comparison indicates that on PTD7, the behavioral sensitivity to SCOP was suppressed in the CVP/S/7/21 group, but it was unchanged in the CPF/S/7/21 group.

Response to pharmacological challenges on PTD21

In this part of the analysis, data obtained on all groups challenged with AMPH or SCOP twice, on PTD7 and PTD21, and only once on PTD21 were taken into consideration.

Amphetamine. The results of AMPH challenge are shown in Fig. 6A. A detailed comparison showed significant effects of both main factors (group effect, $F(5,42) = 3.52$, $p < 0.01$; block effect, $F(1,42) = 27.45$, $p < 0.0001$) as well as significant group • block interaction: ($F(5,42) = 42.53$, $p < 0.0001$). Further comparisons between groups showed significant differences in block 1 ($F(5,168) = 5.55$, $p < 0.0001$) and block 2 ($F(5,168) = 3.69$, $p < 0.005$). CVP-treated groups were responsible for those differences. In the first block, the locomotor activity in group CVP/A/21 was significantly lower than in groups O/A/21 and O/A/7/21, and also in both CPF-treated groups, but in the second block it was significantly lower compared to the O/A/21 and O/A/7/21 groups. In both these blocks, locomotor activity in group CVP/A/21 was lower than 100%, which indicates no response to AMPH. There were no significant differences in the level of locomotor activity between CVP/A/7/21 and CVP/A/21 or even oil-treated groups. In all groups, the levels of locomotor activity in successive blocks decreased gradually. However, only in the oil-treated and CPF/A/7/21 groups, the differences between blocks were significant (locomotor activity in blocks 1 and 2 was significantly higher than in block 4). In order to find out the time course of treatment-induced changes in behavioral responsiveness to AMPH in groups repeatedly challenged with the psychostimulant, locomotor activity in corresponding blocks of measurements performed on PTD7 and PTD21 was compared with use of the Wilcoxon test [20]. It appeared that in group O/A/7/21, differences between the first and second measurement were not significant in blocks 1, 2 and 3, but in block 4 the

activity during the second measurement was significantly higher ($T = 1$, $n = 8$, $p < 0.01$). In group CVP/A/7/21, the postinjection locomotion on PTD21 was significantly reduced compared to that on PTD7 ($T = 1$, $n = 8$, $p < 0.01$ in all comparisons), suggesting a progressive reduction in the sensitivity to AMPH after treatment. Comparison of postinjection measurements on PTD21 showed that in group CVP/A/21 the response to AMPH was in all blocks



AMPH – 1.0 mg/kg; SCOP – 0.75 mg/kg.

Fig. 6. Effects of AMPH and SCOP on the locomotor activity in rats treated with oil, CVP or CPF 21 days after pesticides treatment (after restitution of rbcAChE activity). **A.** Unrelative values (before AMPH challenge) of traveled distance (m) during the 20-min measurement regarded as 100%: O/A/21 – 33 ± 9 , O/A/7/21 – 29 ± 11 , CVP/A/21 – 28 ± 9 , CVP/A/7/21 – 33 ± 7 , CPF/A/21 – 32 ± 8 and CPF/A/7/21 – 30 ± 8 ($n = 8$ in each group). ANOVA: group effect: $F(5,42) = 3.52$, $p < 0.01$, block effect: $F(1,42) = 27.45$, $p < 0.0001$, interaction: $F(5,42) = 42.53$, $p < 0.0001$. * $p < 0.05$ compared to group O/A/21 in the same block, # $p < 0.0001$ compared to block 1 in the same group. The Wilcoxon test: $p < 0.01$ comparisons between groups in response to single (7 days after treatment) and double (7 and 21 days after treatment) AMPH challenge in corresponding blocks. **B.** Unrelative values (before SCOP challenge) of traveled distance (m) during the 20-min measurement regarded as 100%: O/S/21 – 27 ± 6 , O/S/7/21 – 25 ± 7 , CVP/S/21 – 30 ± 6 , CVP/S/7/21 – 25 ± 5 , CPF/S/21 – 28 ± 7 and CPF/S/7/21 – 24 ± 6 ($n = 8$ in each group). ANOVA: group effect: $F(5,42) = 6.77$, $p < 0.0001$, block effect: $F(1,42) = 53.71$, $p < 0.0001$, interaction: $F(5,42) = 40.43$, $p < 0.0001$. * $p < 0.05$ compared to group O/S/21 in the same block, # $p < 0.0001$ compared to block 1 in the same group.

significantly smaller than in group CVP/A/7/21, which may indicate that in group CVP/A/7/21 this progression was hampered. In group CPF/A/7/21, differences were found only in block 1; on PTD21 the postinjection locomotion in this block was significantly higher than on PTD7 ($T = 3$, $n = 8$, $p < 0.05$).

Scopolamine. Figure 6B shows the results of SCOP challenge. Effects of both main factors (group effect: $F(5,42) = 6.77$, $p < 0.0001$; block effect: $F(1,42) = 53.71$, $p < 0.0001$) as well as group $\cdot$ block interaction ($F(5,42) = 40.34$, $p < 0.0001$) were significant. Detailed comparisons showed significant differences between groups in block 1 ($F(5,168) = 6.56$, $p < 0.0001$). In block 1, group O/S/7/21 was significantly more active than the CVP/S/21 group, and group CPF/S/21 was significantly more active than both CVP-treated groups. The comparison between blocks showed that in all groups, except CVP/S/21 and CPF/S/7/21, the level of locomotor activity in block 1 was significantly higher than in blocks 2 and 3. In groups CVP/S/21 and CPF/S/7/21, the differences between blocks were not significant. The low locomotor activity in block 1 may indicate lack of reaction to SCOP-challenge. No significant differences were found between the postinjection locomotion on PTD7 and PTD21 in groups O/S/7/21, CVP/S/7/21, and CPF/S/7/21.

DISCUSSION

The results of the present experiment may be summed up as follows. CVP (1.0 mg/kg) and CPF (70.0 mg/kg) doses did not differ in the magnitude of the treatment-induced rbcAChE inhibition and persistence of this effect; in both cases, the enzyme activity was reduced by about 50% and returned to normal within 14 days. Neither did they differ in the magnitude of the acute behavioral effect: a decrease in spontaneous locomotor activity. It appeared, however, that the used doses of CVP and CPF were not equally potent in: (a) plasma ChE inhibition: CPF inhibited ChE more than CVP; (b) the effect on body weight: only treatment with CPF caused a transient retardation of body weight gain; (c) the efficiency in inducing changes in behavioral sensitivity to AMPH and SCOP, while treatment with CVP resulted in a decreased sensitivity to both these

substances, treatment with CPF appeared practically ineffective. These differences suggest that CVP and CPF differ considerably in the mechanism of action. Therefore, the assumption put forward in the Introduction section that the different effect on the sensitivity to SCOP induced by CPF, observed by Pope et al. [17], and by CVP found in our study [16], was due to differences in the testing procedure is no longer valid. In the present experiment, the effects of CVP, i.e. the decreased behavioral sensitivity to AMPH or SCOP, are consistent with our earlier observations [15,16].

The effects of CPF exposure, however, differ not only from those of CVP, but also from CPF effects observed by other authors. For example, Nostrand et al. [23] did not find differences in the level of inhibition of plasma ChE or rbcAChE 3.5 h and 24 h after oral administration of 60 mg/kg CPF. In addition, both enzymes were inhibited to a higher (90-100%) degree than that measured in our present experiment. It is likely that these differences are due to the fact that rats used in our experiment were of different strain and considerably older than those used by Nostrand et al. [23]. It is known that both genotype [24] and age [25,26] can determine the susceptibility of cholinesterases to inhibition by OPs. It should also be noted that the suggested effect of CPF on body weight finds no support in the literature data. In the present experiment, CPF treatment resulted in retardation of body weight gain for several days. A similar effect was not described in reports from other studies on CPF using doses similar or higher than those applied in our experiment [17,23,24] (Possibly, a decrease was noted, but it was regarded as too obvious to deserve mentioning). The major inconsistency concerns posttreatment alterations in behavioral sensitivity to SCOP. In the study reported by Pope et al. [17], treatment of rats with CPF made them hypersensitive to the locomotor stimulating effect of SCOP. The most likely factors responsible for the absence of a similar effect in our CPF-treated rats were the magnitude of the CPF dose and the exposure route (70.0 mg/kg, i.p.). In the study of Pope et al. [17], CPF was given subcutaneously at a dose of 279 mg/kg, i.e. a fourfold higher than that applied in our experiment. In subcutaneous OP exposure, the development of toxicity

is slower (due to formation of fat OP depots) and lasts longer (due to a gradual OP release from the fat depots to the bloodstream) than in i.p. exposure. There is no doubt that dose and toxokinetics of the given neurotoxin, both may determine the character of the neuroadaptive changes induced by the exposure. The above may explain why in our present experiment the CPF-treated rats did not become hypersensitive to SCOP like the rats treated by Pope et al [17]. The question remains, however, why they did not become, like the CVP-treated rats, hyposensitive to SCOP and AMPH. A transient hyperactivity of the cholinergic brain areas, resulting from inhibition of the brain AChE is the most likely cause of long-term alterations in the behavioral sensitivity to AMPH and SCOP in rats pretreated with CVP. As shown in some of our earlier studies [19,20], in the rat brain, AChE is inhibited by CVP to a similar degree as rbcAChE, which is consistent with reports concerning other OPs. What regards CPF, at present we have no our own data concerning the effect of CPF exposure on the brain AChE. However, other researchers have found that CPF given i.p. to the rat at a dose of 100 mg/kg results in 85.01% inhibition of the brain AChE [27]. One may expect that in CPF dose of 70.0 mg/kg, the inhibition should be proportionally lower and close to that resulting from treatment with 1.0 mg/kg of CVP (i.e. about 50–60%). Thus, the possibility that the different effects of CVP and CPF were related to differences in the level of the brain AChE inhibition is rather vague.

Differences in the interaction with cholinergic receptors might be another likely cause of different effects of CVP and CPF on behavioral sensitivity to AMPH and SCOP. What concerns CVP, some observations suggest that it is neither agonist nor antagonist of muscarinic receptors [28]. As to CPF, there are some data indicating that this pesticide directly interacts with a certain subclass of pre-synaptic M2 receptors (autoreceptors), and the presumable result of this interaction is inhibition of ACh release [2]. Thus, the effect of CPF, but not CVP, on cholinergic receptors may prevent the consequences of AChE inhibition. This supposition is consistent with the recently published observations of Carvajal et al. [29] who found that despite significant AChE inhibition, CPF exposure did not

result in the increase in neural activity in cholinergic brain structures. The above makes it likely that the differences between CVP and CPF effects on the posttreatment sensitivity to AMPH and SCOP are related to differences in the ability to activate the brain cholinergic system(s); high in case of CVP and low in case of CPF.

Apparently discordant with the above supposition is the suppressive effect of pesticide exposure on body weight, which in case of CPF appeared stronger and more persistent than that induced by CVP. It appears, however, from the study of Carvajal et al. [29] that although CPF exposure induces only slight rise in cholinergic activity, it results in significant and delayed activation of cytokine-sensitive brain areas. This suggests an increased synthesis of cytokines, which are known to inhibit food intake and suppress body weight gain [30]. It is then conceivable that the effect of CPF on body weight was related to stimulation of cytokine synthesis and that the CVP efficacy in doing so was limited. Further studies are necessary to solve this issue. The repeated testing procedure applied in the present experiment, although ineffective in identifying the source of differences between the effects of CVP and CPF treatment, provided new, interesting information about the nature of CVP effects. Comparison of data obtained from all groups shows that the effect of CVP treatment on reactivity to AMPH or SCOP varied with time. In case of AMPH, the change – a decrease – was progressive: it was not detectable on PTD7, but well pronounced on PTD21. In case of SCOP, it was detectable on PTD7 and an obvious change in its magnitude occurred only within two weeks later. The progressive reduction in AMPH response after CVP treatment resembles the phenomenon described by Antelmann et al. [31,32] as a time-dependent sensitisation (TDS). It is known that reduced sensitivity to psychostimulants is typical of rats with inborn supersensitivity of the cholinergic system [14]. Therefore, it is possible that exposure to CVP, an indirect cholinergic agonist, sensitized the rat cholinergic system just like exposure to AMPH sensitizes the rat dopaminergic system [33]. If this is so, the behavioral hyposensitivity to AMPH and the reduced response to SCOP after CVP treatment might both be related to adaptive changes within the cho-



linergic system. (Considering the basic mechanism of the CVP toxic activity, this idea sounds quite reasonable). Variation in time courses of the effect might result from different location and possibly from some differences in pharmacological properties between muscarinic receptors mediating the response to SCOP and those contributing to the suppression of the response to AMPH.

Last but not least is the apparent modification of the CVP effect by the AMPH challenge. Comparison of the response to drug challenges on PTD21 in groups challenged twice (on PTD7 and PTD21) suggests that the administration of AMPH, but not SCOP, on PTD7 either slowed down the development of the effect initiated by CVP treatment, or initiated changes opposite in nature to those induced by the pesticide. Whatever the cause, the above observation suggests that the CVP effect on AMPH sensitivity is treatable or "curable".

To sum up, the results of the present research confirm that even a single exposure to organophosphorus pesticides may lead to long-lasting functional alterations within the nervous system. The presence of these alterations is indicated by changes in behavioral response to AMPH or SCOP challenges after treatment. It has been shown that CVP and CPF differ markedly in their ability to induce those changes, which suggests that these two OPs differ in the mechanism of toxicity.

REFERENCES

1. Feldman RG. *Organophosphorus compounds*. In: Feldman RG, editor. *Occupational and Environmental Neurotoxicology*. New York: Lippincott-Raven; 1999. p.421–41.
2. Pope CN. *Organophosphorus pesticides: Do they all have the same mechanism of toxicity?* J Toxicol Environ Health 1999;2:161–81.
3. Eyer P. *Neuropsychopathological changes by organophosphorus compounds – a review*. Hum Exp Toxicol 1995;14:857–64.
4. Lotti M, Becker CE, Aminoff MJ. *Organophosphate polyneuropathy: pathogenesis and prevention*. Neurology 1995;34:658–62.
5. Lotti M, Moretto A. *Promotion of organophosphate induced delayed polyneuropathy by certain esterase inhibitors*. Chem Biol Interact 1999;119–120:519–24.
6. Gershon S, Shaw FH. *Psychiatric sequelae of chronic exposure to organophosphorus insecticides*. Lancet 1961;1:1371–4.
7. Metcalf DR, Holmes JH. *EEG, psychological and neurological alterations in humans with organophosphorus exposure*. Ann New York Acad Sci 1969;160:357–65.
8. Brown MA, Brix KA. *Review of health consequences from high-, intermediate- and low-level exposure to organophosphorus nerve agents*. J Appl Toxicol 1998;18:393–408.
9. Savage EP, Keefe TJ, Mounce LM, Heaton RK, Lewis JA, Burcar PJ. *Chronic neurological sequelae of acute organophosphate pesticide poisoning*. Arch Environ Health 1988;43:38–45.
10. Rosenstock L, Keifer M, Daniel WE, McConnell R, Claypoole K. *Chronic central nervous system effects of acute organophosphate pesticide intoxication*. Lancet 1991;338:223–7.
11. Padilla S. *The neurotoxicity of cholinesterase-inhibiting insecticides: past and present evidence demonstrating persistent effects*. Inhalation Toxicol 1995;7:903–7.
12. Ray DE, Richards PG. *The potential for toxic effects of chronic, low-dose exposure to organophosphates*. Toxicol Lett 2001;120:343–51.
13. Duffy FH, Burchfiel JL, Bartels PH, Gaon M, Sim VM. *Long-term effects of an organophosphate upon the human electroencephalogram*. Toxicol Appl Pharmacol 1979;47:161–76.
14. Overstreet DH, Miller CS, Janowsky DS, Russel RW. *Potential animal model of multiple chemical sensitivity with cholinergic supersensitivity*. Toxicology 1996;111:119–34.
15. Gralewicz S, Lutz P, Szymczak W. *Hyposensitivity to amphetamine following exposure to chlorphenvinphos. Protection by amphetamine preexposure*. Acta Neurobiol Exp 2000;60:203–7.
16. Lutz P, Tomas T, Gralewicz S, Nowakowska E. *Long-term effects of acute exposure to chlorphenvinphos on behavioural responsiveness to amphetamine and scopolamine in rats*. Int J Occup Med Environ Health 2000;13:215–22.
17. Pope CN, Chakraborti TK, Chapman ML, Farrar JD. *Long-term neurochemical and behavioral effects induced by acute chlorpyrifos treatment*. Pharmacol Biochem Behav 1992;42:251–6.
18. Ellman GL, Courtney KD, Andres VJr, Feather-Stone RM. *A new and rapid colorimetric determination of acetylcholinesterase activity*. Biochem Pharmacol 1961;7:88–95.
19. Socko R, Gralewicz S, Gorny R. *Long-term behavioural effects of a repeated exposure to chlorphenvinphos in rats*. Int J Occup Med Environ Health 1999;12:105–17.
20. Gralewicz S, Soćko R. *Effects of a single exposure to chlorphenvinphos, an organophosphorus insecticide, on hot-plate behaviour in rats*. Pol J Occup Med 1989;3:215–22.
21. Winer BJ. *Statistical Principles in Experimental Design*. New York: Mac Graw Hill Book Company; 1962.

22. Siegel S. *Nonparametric Statistics for the Behavioral Sciences*. New York: McGraw-Hill Book Company; 1956.
23. Nostrand AC, Padilla S, Moser VC. *The relationship of oral chlorpyrifos effects on behavior, cholinesterase inhibition, and muscarinic receptor density in rat*. *Pharmacol Biochem Behav* 1997;58:15–23.
24. Gordon ChJ, Watkinson WP. *Strain differences in the laboratory rat: Impact on the autonomic, behavioral and biochemical response to cholinesterase inhibition*. *J Toxicol Environ Health* 1995;45:59–73.
25. Won YK, Liu J, Olivier K, Zheng Q, Pope CN. *Age-related effects of chlorpyrifos on acetylcholine release in rat brain*. *Neurotoxicology* 2001;22:39–48.
26. Shih TM, Penetar DM, McDonough JH, Romano JA, King JM. *Age-related differences in soman toxicity and in blood and brain regional cholinesterase activity*. *Brain Res Bull* 1990;24:429–36.
27. Sachana M, Flaskos J, Nikolaidis E, Hargreaves A, Alexaki-Tzivani-dou E. *Inhibition of rat platelet 5-hydroxydopamine uptake by chlorpyrifos and carbaryl*. *Pharmacol Toxicol* 2001;89:195–200.
28. Gralewicz S, Tomas T, Soćko R. *Interaction of chlorphenvinphos with cholinergic receptors in the rabbit hypothalamus*. *Neurotoxicol Teratol* 1995;17:289–95.
29. Carvajal F, Sanchez-Amate MC, Sanchez-Santed F, Cubero I. *Neuroanatomical targets of the organophosphate chlorpyrifos by c-fos immunolabeling*. *Toxicological Sciences* 2005;84:1–8.
30. Dantzer R. *Cytokine-induced sickness behavior: Mechanisms and implications*. *Ann NY Acad Sci* 2001; 933:222–34.
31. Antelman SM, Caggiula AR, Kocan D, Knopf S, Meyer D, Edwards DJ, et al. *One experience with “lower” or “higher” stressors, respectively, enhances or diminishes responsiveness to haloperidol weeks later: implications for understanding drug variability*. *Brain Res* 1991;566:276–83.
32. Antelman SM, Soares JC, Gershon S. *Time-dependent sensitization – possible implications for clinical pharmacology*. *Behav Pharmacol* 1997;8:505–14.
33. Shannon HE, Peters SC. *A comparison of the effects of cholinergic and dopaminergic agents on scopolamine-induced hyperactivity in mice*. *J Pharmacol Exp Ther* 1990;255:549–53.

