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Behavioural responsiveness to amphetamine or scopolamine following repeated exposure to chlorphenvinphos in rats

Sławomir Gralewicz, Piotr Lutz and **Tadeusz Tomas**

Department of Toxicology and Carcinogenesis, Nofer Institute of Occupational Medicine, 8 Teresy St., 90-950 Łódź, Poland, Email:gralslaw@imp.lodz.pl

Abstract. A number of reports indicate that exposure to organophosphates (OPs), inhibitors of acetylcholinesterase (AChE), may result in long-lasting neurobehavioural alterations suggestive of an increased cholinergic tone. It is known that rats with cholinergic hyperreactivity are behaviourally hyposensitive to cholinergic antagonists and dopaminergic agonists.The purpose of the present study was to find out whether a similar trait would develop in rats exposed to chlorphenvinphos (CVP), an OP pesticide, in the past. The rats were given ten daily i.p. injections of CVP at doses of 0.5 mg/kg (group P-0.5) or 1.0 mg/kg (group P-1.0). The locomotion stimulating effect of i.p. injection of 1.0 mg/kg amphetamine (AMPH), or 0.7 mg/kg scopolamine (SCOP), was assessed on postexposure day 21 (group P-0.5) or 42 (group P-1.0), i.e. after a time sufficient for AChE recovery. The assessment revealed that in group P-1.0 the behavioural response to AMPH and SCOP was significantly depressed. In rats of the P-0.5 group, however, the behavioural response to each of the drugs was increased. The results suggest that, depending on the exposure level, contrasting alterations in some neurotransmitter systems may be induced by repeated exposure to CVP.

Key words: organophosphate, repeated exposure, locomotor activity, amphetamine, scopolamine

INTRODUCTION

Organophosphates (OPs), irreversible inhibitors of acetylcholinesterase (AChE), are widely used for pest control in agriculture and household. Most OP pesticides are assumed to be relatively safe since they do not accumulate in body tissues, and the adverse effects of an overexposure seem to be fully reversible (Ecobichon 1994, Eyer 1995). Exceptions are OPs which inhibit the so-called neuropathy target esterase. Overexposure to these compounds may result in delayed polyneuropathy, an irreversible or only partially reversible condition (Cherniack 1988). There are reasons to suppose, however, that delayed polyneuropathy is not the only possible persisting consequence of exposure to OPs. Numerous clinical and epidemiological observations indicate that occupational or accidental OP exposure may result in neurobehavioural alterations detectable months or even years later (Gershon and Shaw 1961, Metcalf and Holmes 1969, Rosenstock et al. 1991, Savage et al. 1988, Duffy et al. 1979). Several reports from experimental studies on laboratory animals confirm that exposure to OPs may produce effects detectable after restitution of AChE activity. They include changes in some behavioural measures (Ehrich et al. 1993), a decrease in muscarinic receptor function (Tandon et al. 1994), and elevated responsiveness to muscarinic cholinolytics (Pope et al. 1992). In monkeys, an increased beta activity in electrocorticogram (ECoG) was observed up to 1 year after a single symptomatic exposure or a series of subclinical exposures to the OP sarin (Burchfield et al. 1976). The authors presumed that such a change might represent the persistence of the acute OP effects, i.e. elevation in cholinergic activity (Duffy et al. 1979). An increase in the cholinergic tone was also suggested by the character of the behavioural and EEG changes found in rats and rabbits after exposure to chlorphenvinphos [2-chloro-1(2,4-dichlorophenyl) vinyl diethyl phosphate - CVP], an OP pesticide (Gralewicz and Soćko 1997). The changes consisted in an impaired response to novelty, a higher level of the footshock-induced analgesia, and an increased hippocampal cholinergic 4-7 Hz theta response to a stimulus associated with pain. It has been shown that rats characterised by cholinergic supersensitivity are behaviourally hyposensitive to dopaminergic agonists and to cholinergic muscarinic antagonists (Overstreet et al. 1996). If, as we presume, exposure to CVP results in an increased cholinergic tone, then such behavioural hyposensitivity should also characterise rats exposed to this pesticide in the past. Results from our preliminary experiments appeared concordant with the above conjecture; they showed that in rats tested three weeks after a single i.p. exposure to 1.0 mg/kg CVP (1/10 of LD50), the locomotion-stimulating effect of amphetamine (AMPH), an indirect dopaminergic agonist, or scopolamine (SCOP), a muscarinic cholinolytic, was significantly reduced (Lutz et al. 2000). In occupational settings, however, exposure to OPs is rather repeated than single. Therefore, in the present study, the effect of AMPH and SCOP on the rat behaviour in the open field was evaluated in rats after repeated exposures to CVP. Since the most prominent part of behavioural response to a low dose of AMPH or SCOP is an increased forward locomotion (Lanier et al. 1977), only this aspect of the rat behaviour was assessed.

METHODS

Animals

The experiment was performed on male Wistar rats, outbreds, 3-4 months old at the experiment onset. The rats were divided into three main groups, and the main groups were divided into eight subgroups (see Table I). One week before the experiment onset and during the experiment they were housed in single rat cages in standard laboratory conditions (temperature - 22-23^{o}C, humidity 55-60%, light/dark cycle - 12/12 h with light on at 6.00). Standard rat food pellets (Murigran) and tap water were accessible *ad libitum*. All tests were performed between 9.00 and 14.00 h.

Chemicals and the exposure procedure

Chlorphenvinphos (technical grade) was obtained from the manufacturer (Organika-Azot, Jaworzno, Poland). Before use, it was diluted in sterile olive oil. AMPH (d-Amphetamine Sulphate, Sigma) and SCOP (Scopolamine Hydrobromide, Sigma), were dissolved in physiological saline (Natrium Chloratum 0.9%, Polfa). All solutions were administered intraperitoneally at 2.0 ml/kg. CVP was administered during ten exposure days (ED) at daily doses of 0.5 mg/kg (group P-0.5) or 1.0 mg/kg (group P-1.0) within the period of two weeks (5 injections/week). Control animals (group P-0.0) were injected with pure olive oil. After the pre-determined number of postexposure days (PED) animals of respec-

Table I

Groups and the experimental protocol

Group	Subgroup	Number of animals	Exposure (one injection/day, five days a week for two weeks)	Pharmacological testing test drug	postexposure day
P-0.0	P-0.0(A)	8	oil	amphetamine 1.0 mg/kg (i.p.)	21
		9	oil	amphetamine 1.0 mg/kg (i.p.)	42
	P-0.0(S)	9	oil	scopolamine 0.75 mg/kg (i.p.)	21
		9	oil	scopolamine 0.75 mg/kg (i.p.)	42
P-0.5	P-0.5(A)	9	0.5 mg/kg CVP	amphetamine 1.0 mg/kg (i.p.)	21
	P-0.5(S)	9	0.5 mg/kg CVP	scopolamine 0.75 mg/kg (i.p.)	21
P-1.0	P-1.0(A)	9	1.0 mg/kg CVP	amphetamine 1.0 mg/kg (i.p.)	42
	P-1.0(S)	8	1.0 mg/kg CVP	scopolamine 0.75 mg/kg (i.p.)	42

tive subgroups were tested for their behavioural response to AMPH or SCOP. AMPH or SCOP was administered once at 1.0 mg/kg or 0.75 mg/kg, respectively (Table I). The doses were established in pilot experiments. In these experiments, i.p. administration of AMPH at 1.0 mg/kg or SCOP at 0.75 mg/kg induced an overt increase in open-field locomotion with the peak 20-30 min after the injection. The interval between the last CVP dose and the test with AMPH or SCOP was based on the biochemical data from earlier experiments performed at our laboratory (Gralewicz and Soćko 1997). In those experiments, the doses of CVP and the dosing regime were the same as those employed in the present study. The results show that three hours after the last 1.0 mg/kg dose of CVP, AChE activity in blood and brain tissue was reduced by 67-75% and returned to preexposure level within 35 days. In rats receiving CVP daily at 0.5 mg/kg, the respective values were 22-24% and 14 days. Based on the above observations, in the present experiments the interval between the last day of the CVP exposure and the injection of AMPH or SCOP adopted for each pesticide group was a week longer than the time necessary for the AChE recovery, i.e. 21 days in group P-0.5 and 42 days in group P-1.0. The same intervals were adopted for respective control subgroups (Table I).

Apparatus and the test procedure

The "open field" used for assessment of the rat locomotion was a square (100 x 100 cm) arena surrounded by 20 cm high walls. The floor of the arena was painted white. Black crossed lines (borders), 1.0 cm wide, separated the floor into 49 equal squares. The open field was located on the floor of a test room. The conditions in the test room, i.e. temperature, humidity, illumination and the background noise, were the same as in the animal room. For each session, the rat was placed in the open field and an experienced experimenter carefully observed its behaviour for a specified period of time. Locomotion was assessed quantitatively from the number of border oversteps (NBO). The experiment started with three 20 min habituation sessions. Then, the groups were formed. Care was taken to make them as similar as possible in respect of mean body weight, and mean NBO value in the third habituation session. Exposure to CVP started two to three days after the last habituation session. During the exposure, motor activity was measured on ED1 and ED10 in order to assess the acute effect of CVP on locomotion. Each test session was composed of two parts: the preinjection part and the postinjection one. The preinjection testing lasted 10 min. Then the rat was removed from the open field, injected with CVP or oil, and transferred to its home cage. Three hours later, i.e. when the CVP-induced AChE inhibition was supposedly maximal (Kisieliński et al. 1980), the animal was tested again in the open field for 10 min. On PED21 or PED42 the rats were given AMPH or SCOP at the specified test dose. Locomotor activity in the open field was measured for 10 min before and for 50 min after the injection. The postinjection measurement started immediately after the drug administration. For the purpose of the analysis, the NBOs were grouped into six "blocks" numbered from 0 to 5. Block 0 refers to

the NBO from the 10 min preceding the AMPH or SCOP injection, and blocks 1, 2, 3, 4 and 5 refers to successive 10 min sections of the observation period after the injection.

Statistics

Statistical evaluation of the results was performed with the use of a parametric two-way ANOVA (groups x measurements). In case of significant interaction, differences between groups within successive measurements and between measurements within groups were estimated with the use of one-way ANOVA and Tukey's test (Winer 1962). Effects of exposure to CVP on the behavioural responsiveness to AMPH and SCOP were analysed separately.

RESULTS

Effect of exposure on body weight

Figure 1 shows changes in body weight of rats from the first exposure day (ED1) to the 21st postexposure day (PED21). (Of the ten measurements performed during the exposure, only these done on ED1, ED5 and ED10 were taken into account.) The results are presented in relative values; body weight on ED1 has been regarded as 100%. The analysis (groups x measurements) has shown that the effects of both main factors as well as the interaction were significant (group effect: $F_{2,67}$= 40.11, P<0.0001; measurement effect: $F_{1,67}$= 319.58, P<0.0001; groups x measurements interaction: $F_{2,67}$= 64.86, P< 0.0001). As shown in Fig. 1, during the exposure period body weight increased gradually in group P-0.0 (values on ED5 and ED10 significantly larger than on ED1), and remained unchanged in group P-0.5 (differences between ED1, ED5 and ED5 were insignificant). In group P-1.0, body weight decreased sharply during the first half of the exposure period (value at ED5 were significantly lower than that measured on ED1) but started to rise again in the second half of the exposure period. After the exposure, body weight increased gradually in all groups.

Acute effect of CVP on the rat motor activity

The acute effects of the first and the last CVP dose are shown in Fig. 2. Both the main effects and the interaction were significant (group effect: $F_{2,67}$=20.66, P<0.0001; measurement effect: $F_{1,67}$=86.75, P<0.0001; group x measurements interaction: $F_{2,67}$= 30.84, P<0.0001). On ED1, the groups did not differ in the preinjection NBO values. After the injection, the NBO values were significantly lower in all groups. In the pesticide groups, however, the decrease was considerably more pronounced; in both these groups the postinjection NBO values were

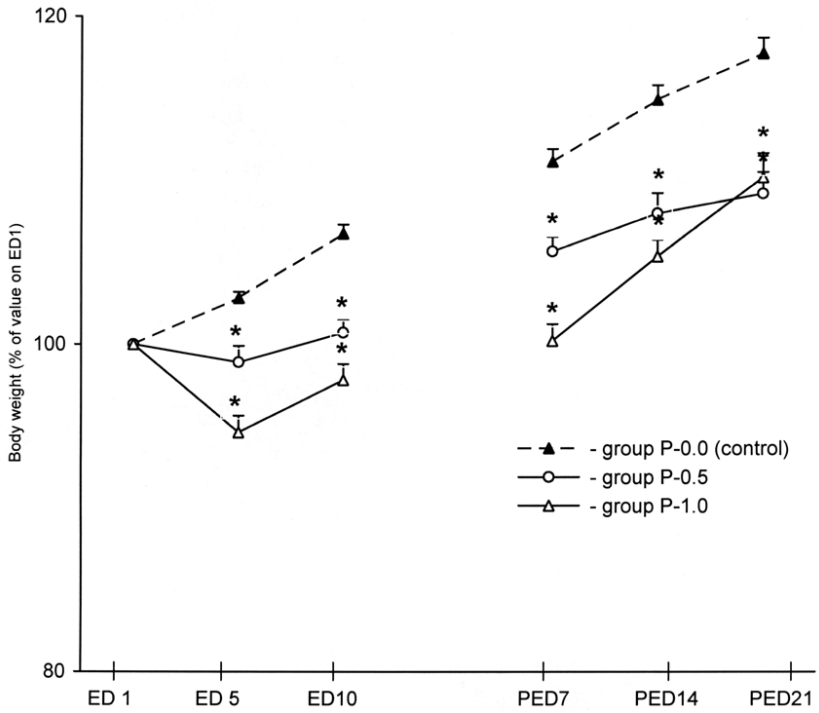

Fig. 1. Changes in body weight of rats in the course of the experiment (mean and SEM). Results of successive measurements are expressed as percent of the body weight on the first day of exposure. Left part of the diagram – measurements during the exposure period. Right part – measurements after the exposure. Measurements done after postexposure day 21 (PED21) are omitted. Black triangles – group P-0.0 (n = 35); open circles – group P-0.5 (n = 18); open triangles – group P-1.0 (n = 17). *, P<0.05 compared to group P-0.0.

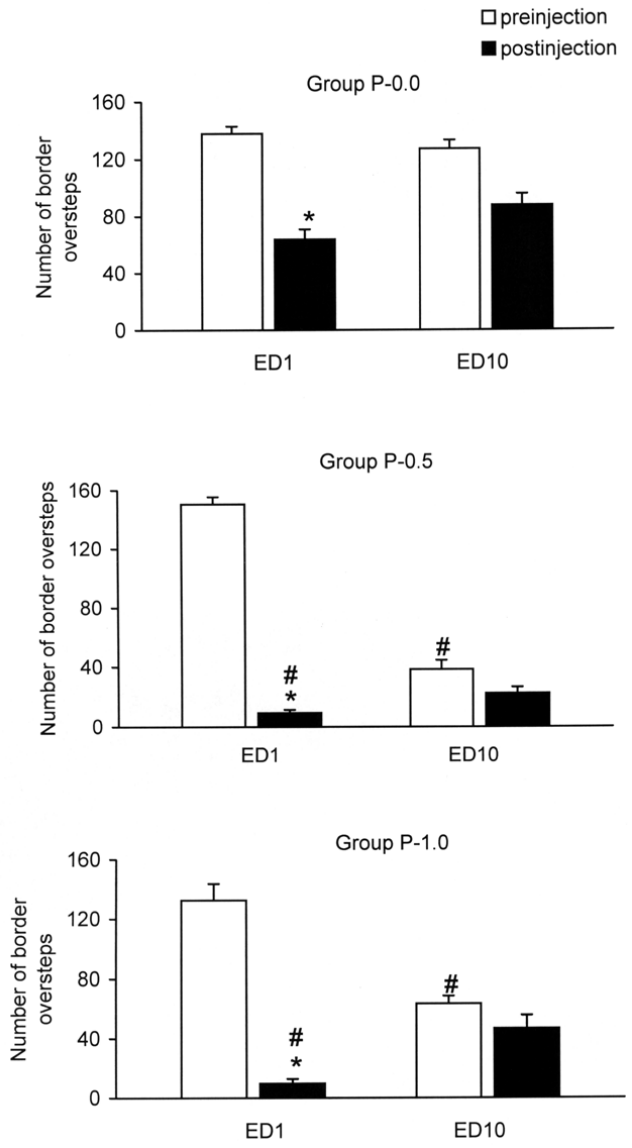

Fig. 2. Diagrams showing effect of the injection of CVP on exposure day 1 (ED1) and exposure day 10 (ED10) on the rat open-field locomotion. The bars denote mean and SEM values of the number of square border oversteps (NBO) during the 10 min measurement done before (white bars) and after (black bars) CVP injection. The postinjection measurement started 3 h after the injection. *, $P<0.05$ compared to preinjection score in the same group; #, $P<0.05$ compared to postinjection score in the P-0.0 group; a, $P<0.05$ compared to preinjection score in P-0.0 group.

significantly smaller than in the P-0.0 group, but group P-1.0 did not differ from group P-0.5. In group P-1.0 and P-0.5, but not in group P-0.0, the preinjection NBO values on ED10 were significantly lower than on ED1. Moreover, on ED10, the preinjection locomotor activity was in both these groups significantly lower than in group P-0.0. After the last injection, the locomotor activity in group P-0.5 and P-1.0 did not differ significantly from that before the injection but was significantly lower than that noted after the injection in group P-0.0. It appears from these comparisons that the CVP exposure resulted in a marked depression of locomotor activity, that this effect was best pronounced 3 h after the first dose but persisted during the whole period of exposure, and that CVP at 0.5 mg/kg was as effective in inducing this effect as at 1.0 mg/kg.

Effect of exposure to CVP on the behavioural responsiveness to AMPH

A preliminary analysis revealed no differences between the control subgroups given the test drugs on PED21 or PED42 . Therefore, for further analysis, they were combined into two subgroups (P-0.0(A), n = 17 and P-0.0 (S), n = 18). As expected, AMPH induced an increase in the rat locomotor activity. However, the groups (P-0.5(A) and P-1.0(A)) differed significantly in the magnitude of this response (Fig. 3). Effects of both main factors as well the interaction were significant (effect of groups: $F_{2,32}$=3.42, $P<0.05$; effect of blocks: $F_{1,32}$=17.32, $P<0.0001$; interaction: $F_{2,32}$=7.74, $P<0.002$). The groups did not differ in respect of the NBO value in block 0. After the injection, however, in block 2 and 3, locomotor activity in group P-1.0 was significantly lower than in group P-0.0 and P-0.5. In block 4 and 5, no significant differences between groups were noted. Comparisons between blocks within each group showed significant differences in group P-0.0(A) ($F_{5,160}$=11.03, $P<0.0001$) and in group P-0.5(A) ($F_{5,16}$0=8.39, $P<0.0001$). In both these groups, the NBO number in block 3 and 4, and in group P-0.5(A) in block 5 was significantly higher than in block 0. In group P-1.0(A) the analysis revealed no significant differences between successive blocks ($F_{5,160}$=1.77, $0.1<P<0.2$). It needs emphasising that the evidently weaker locomotor response to AMPH in group P-1.0 was not paralelled by an overt increase in other movement patterns incompatible with locomotion.

Effect of CVP exposure on the behavioural responsiveness to SCOP

Injection of SCOP, like injections of AMPH, caused a marked increase in locomotor activity. As shown in

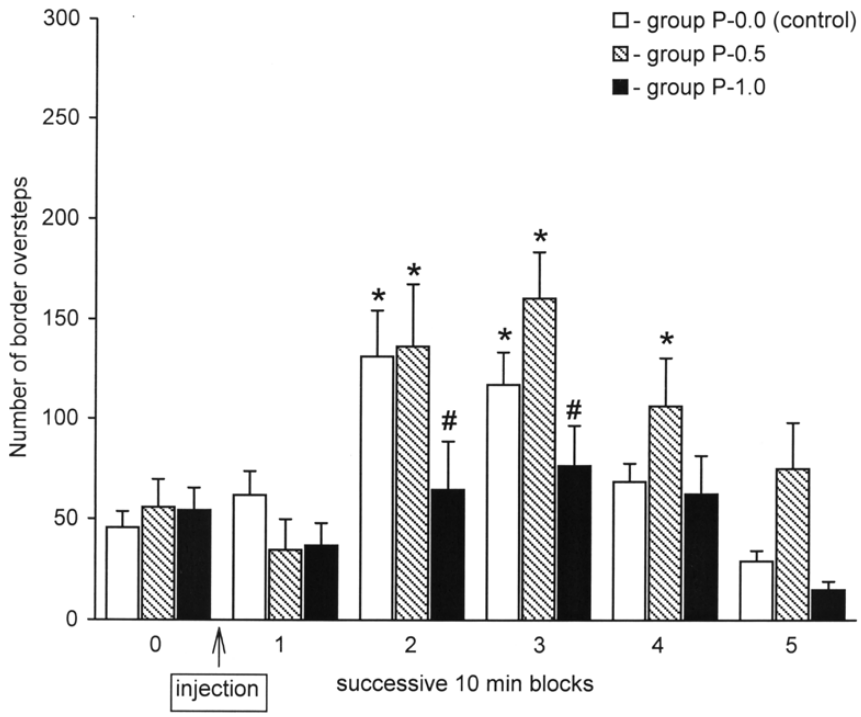

Fig. 3. Effect of AMPH (1.0 mg/kg, i.p.) on the open-field locomotor activity in rats of the P-0.0 group (white bars), P-0.5 group (striped bars), and P-1.0 group (black bars). Locomotor activity was measured for 10 min before AMPH injection (block 0) and for 50 min after the injection (blocks 1 – 5). Bars represent means and SEM . *, $P<0.05$ compared to corresponding block in group P-0.0; #, $P<0.05$ compared to block 0 in the same group.

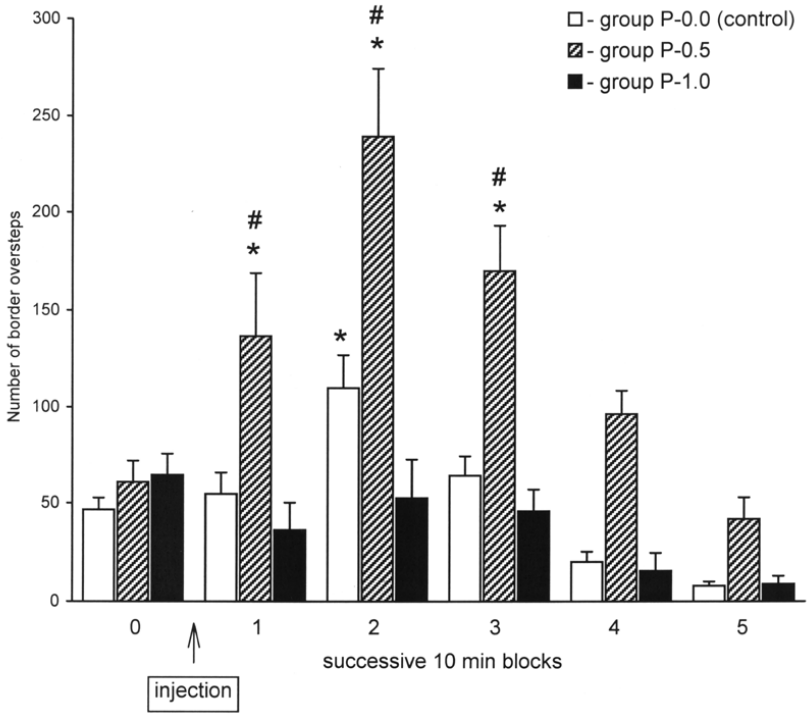

Fig. 4. Effect of SCOP (0.75 mg/kg, i.p.) on the open-field locomotor activity in rats of the P-0.0 group (white bars), P-0.5 group (striped bars), and P-1.0 group (black bars). The description as in Fig. 3.

Fig. 4, the groups differed markedly in respect of the magnitude of the response to SCOP (effect of groups: $F_{2,32}$=24.65, P<0.0001; effect of blocks: $F_{1,32}$=28.70, P<0.0001; interaction: $F_{2,32}$=21.21, P<0.0001). In block 0 and 5 no differences between groups were recorded. In the remaining 10 min blocks, the NBO value in group P-0.5(S) was significantly higher than in groups P-0.0(S) and P-1.0(S). Comparisons within groups showed significant differences between blocks in group P-0.0(S) ($F_{5,160}$=12.08, P<0.0001) and in group P-0.05(S) ($F_{5,160}$=25.21, P<0.0001), but not in group P-1.0(S) ($F_{5,160}$=2.02, 0.05<P<0.10). In group P-0.0(S), the response to SCOP was relatively short; only in block 2 it was significantly higher than in the remaining 10 min blocks. In group P-0.5(S) the locomotor activity in blocks 1, 2 and 3 was significantly higher than in the remaining blocks, indicating that in this group the response to SCOP lasted much longer and appeared after a shorter latency than in group P-0.0. In group P-1.0, differences between blocks were insignificant, indicating a decreased behavioural responsiveness to SCOP.

DISCUSSION

In the present experiment, repeated exposure of rats to CVP at daily doses of 1.0 mg/kg suppressed spontaneous locomotion, reduced body weight, and resulted in a behavioural hyposensitivity to AMPH or SCOP given six weeks after the last CVP dose. Exposure at doses of 0.5 mg/kg appeared equally efficient in suppression of spontaneous locomotion as that at 1.0 mg/kg, retarded transiently body weight gain, and resulted in behavioural supersensitivity to the locomotion stimulating effect of AMPH or SCOP three weeks after exposure discontinuation.

Suppression of spontaneous locomotion and decrease in body weight are typical effects of exposure to OPs. The effect on body weight noted in the present experiment was apparently dose-related, whereas that on the open-field locomotion was not. Locomotor activity is certainly more sensitive to CVP than body weight, and the above result may indicate that the doses of 0.5 mg/kg were sufficiently high to produce the maximum effect on this measure.

It is worth noting that by the end of the exposure period a reduction in the effect of CVP on locomotion and on body weight was evident in rats of both exposed groups. Development of tolerance to OPsupon repeated exposure as well as the mechanism of this phenomenon are well known (Russel et al.1986) and require no further discussion. In contrast to the effects specified above, the opposed changes in the behavioural response to AMPH and SCOP, namely an increase in group P-0.5 and a decrease in group P-1.0, are quite surprising. To our knowledge, such bi-directional effects of exposure to OPs have not been reported in the relevant literature. In two studies (Buschnell et al. 1993, Pope et al. 1992), the authors found an increased behavioural responsiveness to SCOP after exposure to chlorpyrifos, a widely used OP pesticide. In Pope et al. (1992) study, this effect persisted after the AChE activity and muscarinic receptor density in striatum and cortex had returned to normal level. The present experiment differed from that of Pope et al. in respect of the exposure conditions (repeated, intraperitoneal vs. single, subcutaneous). Moreover, the inhibition of AChE activity by the single 279 mg/kg dose of chlorpyrifos was more severe (<90%) and lasted longer (at least six weeks) than that produced by ten daily 0.5 mg/kg doses of CVP (less than 25% for less than four weeks including the exposure period (Gralewicz and Soćko 1997)). It seems quite likely, however, that the same mechanism was responsible for the elevated responsiveness to SCOP noted in the Pope et al. (1992) study and in Group P-0.5 of the present experiment. This issue will be discussed later. The P-0.5 and P-1.0 groups of the present experiment differed in two procedural factors: the daily CVP dose and the duration of the postexposure interval (21 or 42 days). Theoretically, each of these factors might affect the rat responsiveness to AMPH or SCOP. However, in case of the 1.0 mg/kg CVP dose, depressed behavioural responses to AMPH or SCOP were noted on PED21 (Gralewicz et al. 2000, Lutz et al. 2000) and on PED42 (present experiment). It might mean that the time factor did not count considerably, and that the key factor was the daily CVP dose. As mentioned in the introduction, rats with hyperactive cholinergic system are characterized by behavioural hyposensitivity to cholinergic antagonists and dopaminergic agonists (Overstreet et al. 1996). Thus, the presence of this trait in rats of the P-1.0 group is consistent with the supposition that a persistent cholinergic hyperactivity may constitute one of the consequences of exposure to CVP. The opposite trait in group P-0.5, however, suggests that in this group the neuroadaptive changes were contrary to those observed in group P-1.0.

We have found no reports in the relevant literature indicating that, depending on the applied dose(s), expo-

sure to OPs could produce contrasting neurobehavioural effects detectable long after exposure cessation. However, antagonistic changes in the behavioural responsiveness to a test drug were observed in rats by Antelman et al. (1991) weeks after low or high level exposures to a variety of chemical or physical stimuli. According to these authors, stressfulness was the factor determining the ability of a stimulus to induce such long-lasting behavioural effects and the character of these effects was determined by the magnitude of the non-specific stress response induced by the exposure (Antelman et al. 1991, 1997). The acute behavioural and EEG symptoms (Gralewicz and Soćko 1997) and the rise of plasma corticosterone levels (Osicka-Koprowska et al. 1984) which may be induced by CVP leave no doubt that this pesticide is a stressful agent. In the present experiment, judging from the changes in the body weight, exposures to CVP at 0.5 mg/kg were less stressful than exposures at 1.0 mg/kg. Thus, one may presume, following the Antelman et al. suggestions quoted above, that the exposure-related unspecific stress response was the primary cause of the changes in sensitivity to AMPH and SCOP after exposure to CVP and that the direction of these changes in groups P-0.5 and P-1.0. was determined by the magnitude of this response. The above conjecture might help explaining why, in the present experiment, an effect similar to that produced by chlorpyriphos in the Pope et al. study (Pope et al. 1992), i.e. increased behavioural sensitivity to SCOP, occurred in the rats of the P-0.5 group, but not in the rats of the P-1.0 group. As Pope et al. noted, in their experiments the applied dose of chlorpyrifos (279 mg/kg. s.c.) resulted in a more than 90% AChE inhibition, but "diarrhoea was the only relatively consistent sign of toxicity". CVP given at a dose inhibiting AChE activity by more than 90% induces "cholinergic crisis" (experimenter's personal observations). A single injection of CVP at the 1.0 mg/kg dose, although inhibits AChE activity only by about 50% (Gralewicz and Soćko 1997), induces typical, albeit moderate, acute symptoms (crouching, tremor, salivation). At the 0.5 mg/kg dose (AChE inhibition less than 25%), CVP produces no overt symptoms except for a decrease in spontaneous locomotion. It is then quite likely that, in terms of acute toxicity and the potential to induce stress response, of the two CVP doses, 0.5 mg/kg or 1.0 mg/kg, the former is a better match for the 279 mg/kg dose of chlorpyrifos. It may account for the similarity in the effect on the behavioural responsiveness to SCOP in rats of the P-0.5 group and rats in the experiment of Pope et al. (1992). The possibility that the changes in behavioural responsiveness to AMPH and SCOP following CVP exposure could be related to the unspecific stress response, and the fact that exposure level can determine the direction of these changes point out new perspectives for further research. First, it is quite likely that effects similar to those of CVP could be produced by multitude of other "stressors". Second, the opposed changes in the response to AMPH and SCOP following low and high level exposures suggest that opposed neuroadaptative alterations have developed in the CNS. It means that a low-level exposure to a "stressor" in the past may protect against effects of a future high level exposure to the same or another stressor. Both these issues are worth being investigated experimentally.

CONCLUSIONS

Repeated i.p. exposure of the rat to CVP can make the animal behaviourally hypersensitive or hyposensitive to AMPH and SCOP given weeks after the exposure cessation. The type of the effect depends on the daily dose of the pesticide: exposure to low doses (0.5 mg/kg) leads to hypersensitivity, whereas exposure to moderate doses (1.0 mg/kg) causes hyposensitivity to AMPH and SCOP. It is assumed tentatively that the alterations in the behavioural responsiveness to AMPH and SCOP were causally related to the non-specific stress response produced by the exposures.

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