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LONG-TERM EFFECTS OF ACUTE EXPOSURE TO CHLORPHENVINPHOS ON BEHAVIOURAL RESPONSIVENESS TO AMPHETAMINE AND SCOPOLAMINE IN RATS

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Abstract. We investigated the effect of acute exposure to chlorphenvinphos (CVP) (2-chloro-1 (2,4-dichlorophenyl)-vinyl-diethyl-phosphate), an organophosphate anticholinesterase, on the amphetamine- and scopolamine-induced open-field locomotion in Wistar rats. CVP was administered at a single i.p. dose of 1.0 mg/kg (1/10 of LD_{50}). In part of the rats cholinesterase (ChE) was determined. Three hours after CVP injection, the ChE activity decreased by about 27%. It returned to the preinjection level within 14 days after the exposure. In the behavioural part of the experiment, the animals were challenged with 1.0 mg/kg amphetamine or 0.75 mg/kg scopolamine three weeks after CVP exposure, i.e. after a period of time sufficient for cholinesterase recovery. It has been found that in the CVP-exposed rats, the behavioural responses to amphetamine or scopolamine challenge (the increase in locomotor activity) was significantly reduced compared to the controls. This suggests that acute exposure to CVP produced an increase in cholinergic activity which persisted long after ChE activity had returned to normal.

INTRODUCTION

Chlorphenvinphos (CVP) (2-chloro-1(2,4-dichlorophenyl)-vinyl-diethyl-phosphate) is an active component of many pesticide preparations. It is produced and used in Poland. Toxic properties of CVP, like of other organophospohorous compounds (OPs), comprise inhibition of acetylocholinesterase (AChE), the enzyme responsible for the decomposition of acetylcholine (ACh), one of the main neurotransmitters in the central nervous system (CNS). Clinical studies and epidemiological data point to long-term disturbances in the CNS of people with a history of acute or chronic exposure to some OPs. The major symptoms include concentration/attention and memory problems, depressive states, fatigue, irritability and

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sleep disorders (7,18,20,21). The neurophysiological basis of these long-term effects (of OPs exposure) has not as yet been elucidated. Some authors (4,14) suggest that exposure to OPs may increase the sensitivity of CNS cholinergic structures. In our previous experiments on the rat acutely exposed to CVP, we observed an impaired response to novelty, a higher level of the footshock-induced analgesia and an increase in hippocampal 4-7Hz theta response to a stimulus signalling pain. These changes could be attributed to an increased cholinergic activity (9,10). Overstreet et al. (14) show that some inbred rats characterised by an overactive cholinergic system exhibit behavioural subsensitivity to: scopolamine (SCOP) – a muscarinic cholinergic antagonist, and apomorphine – a direct dopaminergic D1/2 agonist, or amphetamine (AMPH) – an indirect dopaminergic D1/2 agonist. Their acute exposure to CVP produced an increase in the cholinergic activity, which persisted long after ChE activity had returned to normal. A similar behavioural hyposensitivity might be expected in the CVP exposed rats, tested after a period of time sufficient for a full ChE recovery.

The purpose of our present experiment was to verify these conjectures. An increased locomotor activity constitutes the major behavioural response to low-dose AMPH (19) or SCOP injection (16). The magnitude of this response to AMPH or SCOP was assessed by the open-field test. Only this aspect of the rat behaviour was investigated in our present study.

MATERIALS AND METHODS

Animals

Behavioural and biochemical tests were performed on 69 male, 3–4 months old, with body weight of 270–330 g, outbred Wistar rats from our Insitute's breeding farm. They were housed in single rat cages, at standard laboratory conditions (21–23°C and 55%–60% relative humidity, the light/dark cycle was 12/12 h; standard rat food pellets and water were accessible *ad libitum*). Behavioural and biochemical tests were performed between 9 a.m. and 2 p.m.

Chemicals

CVP (technical grade, Jaworzno) diluted in sterile oil, amphetamine (d-Amphetamine sulfate, Sigma) and scopolamine (Scopolamine Hydrobromide, Sigma), both dissolved in physiological saline (Natrium Chloratum 0.9, Polfa), were administered.

Methods

In biochemical and behavioural tests of our experiment, control groups were given a single intraperitoneal (i.p.) injection of sterile olive oil and the exposed groups received one 1.0 mg/kg dose of CVP (1/10 LD_{50}) diluted in sterile oil. I.p. injection is different from human exposure, but we used this route to make it sure that after exposure maximal effect of CVP in whole blood (ChE activity) could be observed. Especially that i.p exposure to CVP at this dose produced only mild non-specific effects on the morphological and enzymatic structures of the liver (12). It is essential that animals used in behavioural tests be in good condition. In each group, the volume of the injected liquid was about 2.0 ml.

In behavioural part, control solution saline was given via the same route. In pilot experiments we tested 0.5, 1.0 and 5.0 mg/kg doses of AMPH and 0.5, 0.75, and 1.0 mg/kg doses of SCOP. We used doses: 1 mg/kg AMPH and 0.75 mg/kg SCOP. At these doses, over twofold increase in open-field locomotor activity was observed 20–30 min after injections.

Biochemical tests

Biochemical tests – changes in cholinesterase (ChE) activity – were performed on 12 rats divided into two groups (6 animals in each group), each animal was treated with oil or CVP (Table 1). ChE activity was determined in peripheral blood drawn from the tail vein into heparinized glass test tube. ChE may be regarded as a model of AChE in the nervous system. The enzyme activity seems useful as an indicator of the effects of the AChE inhibitors, such as pesticides (11). In our experiment ChE activity was determined: seven days before exposure to CVP or oil (control), as well as 3 h, 14 days and 21 days after the exposure. The measurements of ChE activity were performed colorimetrically (6).

Table 1. Changes in the whole blood ChE after exposure to a single dose of oil and after exposure to a single dose of 1.0 mg/kg CVP in rats used for biochemical tests

Group	Before treatment	After treatment		
	7 days	3 h	14 days	21 days
Treated with oil n = 6	705.0±50.9	595.0±47.7	646.6±43.7	700.0±36.6
Treated with CVP n = 6	730.0±73.5	531.6±47.2*	680.0±42.2	706.6±31.7

* p = 0.05 compared to the pre-treatment result.

Behavioural tests

In the behavioural part, 57 rats were used. They were divided into 6 groups (Table 2). For testing the locomotor activity, the rat was placed in the centre of a square (100 × 100 cm) arena (open-field) surrounded by 20-cm high walls with the surface divided into 49 equal squares by lines painted on it, and observed for 10 min. The number of square borders crossed (NoSBC – locomotor activity) was recorded

Table 2. Group and sequence of injections in behavioural tests

Groups	1st injection	2nd injection 21 days after 1st injection	Number of animals
GROUP O/S	Oil	Saline	10
GROUP P/S	CVP	Saline	10
GROUP O/A	Oil	Amphetamine	10
GROUP P/A	CVP	Amphetamine	9
GROUP O/Scop	Oil	Scopolamine	9
GROUP P/Scop	CVP	Scopolamine	9

manually by the experimenters. The whole testing consisted of four (two pre- and two post-injection) 10 min sessions. After the pre-injection testing, the rat was removed from the open-field, injected in adjacent room and then transferred to its home cage. The testing began 3 h after the first injection (CVP or oil), and 20 min after the second injection (AMPH or SCOP). Based on the results of biochemical assays, the rats were challenged with AMPH or SCOP 21 days after CVP or oil injections.

Statistical analysis

The results of the ChE activity measurements were analysed by parametric and non-parametric two-way analysis of variance (ANOVA). The number of square borders crossing values was analyzed by a two-way parametric ANOVA.

RESULTS

Biochemical results

The two-way parametric ANOVA (group x measurements) revealed no significant effects between the group treated with oil and the group treated with CVP in control measurements. The non-parametric two-way Friedman ANOVA revealed differences between the groups. In the group treated with CVP, ChE activity was significantly lower 3 h after the injection ($\chi^2 = 7.4$ df $= 3$, $p = 0.05$) compared to measurements before CVP injection. In this group, enzyme activity returned to normal within 14 days after the exposure ($\chi^2 = 5.25$ df $= 3$, no significant differences). There were no significant differences in ChE activity in the group treated with oil before and after injections (Table 1).

Behavioural observations

Control animals of all groups behaved similarly. After entering the open-field, the rats showed evident interest in new surrounding, demonstrated by an increased locomotor activity, sniffing of new stopping places and alternative rearings. The higher locomotor activity during the first minutes of observation gradually decreased and toilet episodes prolonged. Finally, some of the rats, chose one of the open-field corners and stopped there. Three hours after CVP exposure, the rats showed high increase in mobility and the gait was disturbed. Tremor of hind limbs and piloerection was quite characteristic. Generally, 24 h after exposure the rats behaved normally in their home cages. In rats treated with oil, an acute injection of 1 mg/kg AMPH or 0.75 mg/kg SCOP produced an increased incidence of forward locomotion, head movements, toilet episodes, sniffing and rearing. In CVP treated groups, these effects were not observed.

Behavioural results

The spontaneous locomotor activity (NoSBC) in the open-field test was subjected to the statistical analysis by two-way ANOVA (group x measurement, parametric test). The effects of both main factors were significant: effect of groups ($p < 0.001$), effect of measurement ($p < 0.001$), interaction ($p < 0.001$). The comparisons between measurements revealed no significant differences in pre-injection NoSBC indices of measurements 1 and 3 (no effect of oil/CVP injection on spontaneous locomotor activity).

Significant differences were observed only in the post-injection locomotor activity: measurement 2 ($p < 0.001$) and measurement 4 ($p < 0.001$). The CVP injection (P/S, P/Scop and P/A) produced significant decrease in locomotor activity compared to that in O/S, O/A and O/Scop groups. After the second injection, no significant difference between O/S and P/S groups was found. P/A ($p < 0.001$) group showed significantly lower locomotor activity than O/A group, and P/Scop ($p < 0.001$) scored significantly lower locomotor activity than O/Scop group. In measurement 4 there was no significant difference between P/S and P/Scop groups. However, group P/A showed significantly higher locomotor activity compared to P/S and P/Scop, and significantly lower locomotor activity compared to group O/A. (Fig. 1).

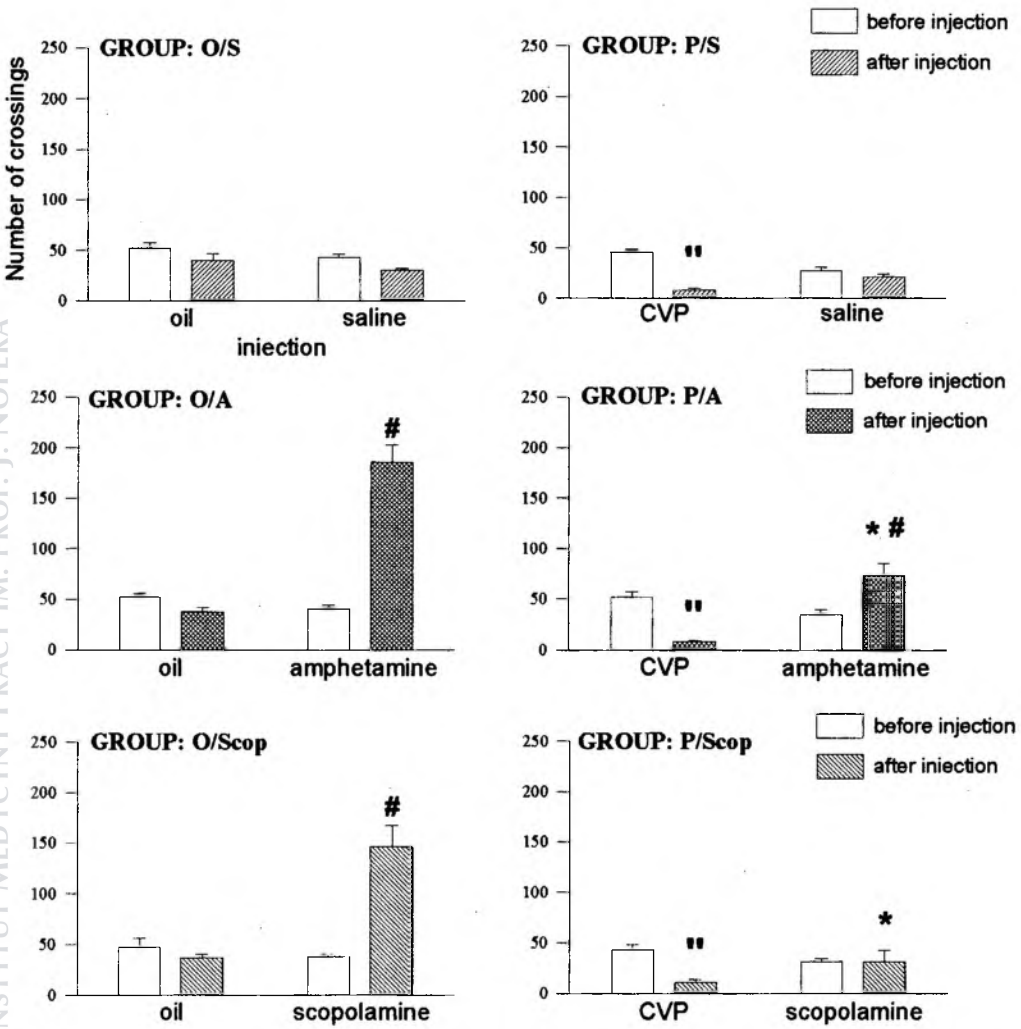

Fig. 1.
"$p < 0.001$ compared to locomotor activity after the 1st injection between all groups, *$p < 0.001$ compared to locomotor activity after the 2nd injection, O/A–P/A and O/Scop–P/Scop groups, # $p < 0.001$ compared to locomotor activity after the 2nd injection between O/S–O/A–O/Scop and P/S–P/A–P/Scop groups.

DISCUSSION

As shown by the results of the biochemical part, i.p. administration of CVP at a single 1.0 mg/kg dose resulted in about 27% decrease in blood ChE activity three hours after dosing, and the enzyme activity returned to the pre-exposure level within 14 post-exposure days.

There were no significant differences in ChE activity in the group treated with oil and CVP 3 h after the injection. Both these observations are concordant with the results obtained in our earlier CVP studies (8,9). They indicate that in the exposed rats used in the behavioural part of the present experiment, the level of ChE activity in blood, and most likely in the brain (9) was normal at the time of AMPH or SCOP administration. Yet, the behavioural response to each of the two challenges was significantly reduced in these animals. The character of the behavioural and EEG effects of CVP exposure observed in earlier studies (8,9,10) suggested an increased cholinergic reactivity. In the light of the observations, made by Overstreet et al., (14) mentioned in the Introduction section, the decreased behavioural responsiveness to AMPH, an indirect dopaminergic agonist, or SCOP, direct muscarinic blocker, might be expected if the cholinergic system was hyperreactive. The dopaminergic and cholinergic systems play an important role in the control of CNS locomotor activity. The antagonism between these systems in locomotor behaviour (3) precludes a simple answer to the question based on the results of any behavioural study. Both systems, cholinergic and dopaminergic, are involved in the mediation of the acute effects of OPs exposure (2), and long-term neuroadaptive changes following the exposure are likely to occur in both these systems. Thus, the previous and the present results suggest that even a single administration of CVP at a relatively low dose may result in an increased cholinergic tone, outlasting the decrease in ChE activity. Several questions may be asked regarding the results of the present study. First, it would be interesting to find out whether and how the reduced behavioural responsiveness to AMPH or SCOP after the ChE recovery is related to the level of ChE inhibition (i.e. CVP dose) produced by the exposure. An experiment is in progress which, as we hope, will give an answer to this question. Second, and the most important question is whether the effects similar to those described in the present study could be produced by exposure to other OPs. The OP compounds in current use differ with respect to the affinity to AChE (and other esterases) and ability to interact with cholinergic receptors (17). It is likely then, that they may differ with respect to the long-term neuroadaptive changes triggered by the exposure. However, there are just a few reports on the persisting effect of OPs from animal studies (14), which limits the possibility of comparisons. In one of these studies (16), the effect of an acute exposure to chlorpyrifos (CPF), a commonly used OP pesticide, on the behavioural responsiveness to SCOP was investigated. The authors have found out that in the CPF exposed animals, unlike in our CVP exposed rats, the SCOP-induced locomotor response was increased 12 weeks after the exposure, when both the AChE activity and muscarinic receptor density in the brain returned to normal levels. In another study on the same OP, no effects on behavioural responsiveness to AMPH were noted (1). The discrepancy between our findings and those obtained by Pope et al. (16) may result from differences in the testing procedures. In our experiment, the rats were administered SCOP once, whereas they challenged them with SCOP repeatedly at different time after the exposure.

It cannot be excluded then that Pope et al. tested finally the effect of a combined exposure (acute exposure to CPF plus intermittent exposure to SCOP). Differences in the duration of AChE depression after the exposure may be another cause of the discrepancy. In the study reported by Pope et al., the recovery of ChE activity after the subcutaneously CPF exposure lasted about 12 weeks. Suppression of ChE activity for such a long time might result in the neuroadaptation qualitatively different from that developing during a two-week period of reduced enzyme activity noted in our study. Differences in the neuroadaptation following CPF and CVP exposures may also be due to differences in the mode of interaction with cholinergic receptors. Some data indicate that CPF (CPF oxon) binds reversibly to a subpopulation of muscarinic receptors in the rat striatum (13,16). There is considerable evidence for the possible direct interaction between organophosphorus esters and targets such as muscarinic (mAChR) and nicotinic (nAChR) acetylcholine receptors. Some mechanisms are proposed by which organophosphorus ester increases brain tissue levels of cyclic AMP and cyclic GMP, mediated through ACh-related actions on receptors and the release of catecholamines. The question has been raised as to whether or not these second messenger effects are due to the ACh-like action or to some other, as yet unidentifield, direct mechanisms (5). On the other hand, CVP injected into the rabbit hypothalamus neither induces any EEG or behavioural response suggestive of muscarinic stimulation, nor prevents induction of such a response by a subsequent injection of oxotremorine, a muscarinic agonist (10). This suggests that CVP, unlike CPF, does not interact directly with muscarinic receptors. A comparative study seems to be the best way to find out whether the differences between our results and those reported by Pope et al. (16) were due to differences in the methodology or to differences in the toxic properties of the compounds used. We plan to perform such a study in the near future.

To sum up, the results of the present experiment confirm that exposure to CVP may result in long-term functional alterations in the rat CNS. They also strongly support the supposition that these alterations may consist in an increased cholinergic reactivity. Further studies should reveal whether exposure to other OP anticholinesterases could produce effects similar to those produced by exposure to CVP. Results of such studies may help understanding the nature of at least some of the complaints and symptoms reported by people with the history of past OPs exposure (19).

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