ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 2000, 60: 203-207

Hyposensitivity to amphetamine following exposure to chlorphenvinphos - protection by amphetamine preexposure

Sławomir Gralewicz,[1] Piotr Lutz[1] and **Wiesław Szymczak[2]**

[1]Department of Toxicology and Carcinogenesis and [2]Department of Epidemiology, The Nofer Institute of Occupational Medicine, 8 Teresy St., 90-950 Łódź, Poland, Email:gralslaw@imp.lodz.pl

Abstract. We investigated the effect of an acute exposure to chlorphenvinphos (CVP), an organophosphate anticholinesterase, on amphetamine-induced open-field locomotion in rats. CVP was administered in a single i.p. dose of 1.0 mg/kg (1/10 of the LD_{50}). All animals were challenged with 1.0 mg/kg amphetamine (AMPH) three weeks after the CVP exposure, i.e. after a time sufficient for acetylcholinesterase recovery. Some rats were also given AMPH three weeks before the CVP exposure. In rats challenged with AMPH only once after the CVP exposure, AMPH- induced open-field locomotion was significantly reduced. Such an effect was not observed in rats given AMPH three weeks before the CVP exposure. The results suggest that a single CVP exposure may result in persistent dopaminergic hyposensitivity, and that an amphetamine pretreatment may protect the rat against this effect.

Short communication

Key words: organophosphate pesticide, acute exposure, amphetamine challenge, rat

Organophosphate compounds (OPs), irreversible inhibitors of acetylcholinesterase (AChE), are widely used as insecticides. Several studies have revealed neurobehavioral disturbances (Gershon and Shaw 1961, Metcalf and Holmes 1969, Savage et al. 1988, Rosenstock et al. 1991, Yokoyama et al. 1998) and EEG changes (Duffy et al. 1979) in persons exposed acutely or repeatedly to OPs. The most consistent complaints and symptoms include concentration/attention and memory problems, tiredness, depressive states, sleepiness, and irritability. The neurophysiological bases of these long-term effects of OP exposure are not known. According to some authors, OP exposure may result in a persistent increase in sensitivity of cholinergic structures in the CNS (Duffy et al. 1979, Overstreet et al. 1996). To date, however, direct confirmation of this supposition from controlled animal studies is lacking.

In our previous studies we investigated neurobehavioral effects of exposure to chlorphenvinphos [2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate - CVP], an OP pesticide, on rats and rabbits. The animals were exposed to CVP acutely or repeatedly and tested after a time sufficient for brain and erythrocyte AChE recovery. The observed effects were an impaired response to novelty, a higher level of the footshock-induced analgesia, and an increased hippocampal 4-7 Hz theta response to a stimulus associated with pain. These could be accounted for by increased cholinergic activity (Gralewicz and Soćko 1997).

It has been found that inbred rats characterized by an overreactive cholinergic system show behavioral hyposensitivity to dopaminergic agonists (Overstreet et al. 1996). If exposure to CVP resulted in an increase in cholinergic activity outlasting the depression of AChE, a similar hyposensitivity might be expected in rats exposed to this pesticide and tested after a time sufficient for AChE recovery. The purpose of the present experiment was to find out whether and how the rat behavioral responsivenes to amphetamine (AMPH), an indirect dopaminergic agonist (Jackson and Westlind-Danielsson 1994, Jones et al. 1998), is altered by acute exposure to CVP. In the rat, the most prominent part of behavioral response to a low dose of AMPH is an icreased forward locomotion (Lanier and Isaacson 1977). Therefore, only this aspect of rat behavior was assessed in the present study.

The experiment was performed on 39 male Wistar rats, 3-4 months old, and weighing 285-320 g at the onset of the experiment. The animals were divided into four groups (see Table I). During the experiment they were housed in single rat cages at 22°C, with a light/dark cycle of 12/12 h (light on at 0600 h). Standard rat food pellets (Murigran) and tap water were accessible *ad libitum*. AMPH (d-amphetamine sulphate, Sigma), dissolved in physiological saline, or CVP [2-chloro-1-(2,4-dichlorophenyl) vinyl diethyl phosphate, technical grade, Jaworzno], diluted in sterile olive oil, both were administered intraperitoneally. Control solutions, saline or olive oil, were given via the same route. In each case the volume of the injected fluid was 2.0 ml/kg of body weight. CVP was administered at the 1.0 mg/kg dose (1/10 LD_{50}). According to our earlier studies (Gralewicz and Soćko 1997), a single i.p. injection of CVP at that dose inhibits erythrocyte and brain AChE by about 50%. The enzyme activity returns to normal levels within 14 postexposure days. Based on this information, a three-week interval between the CVP injection and the AMPH challenge was adopted to make sure that at the time of AMPH injection the AChE activity was not depressed. AMPH was administered at a dose of 1.0 mg/kg. In a pilot experiment, administration of AMPH at that dose resulted in a more than twofold increase in ambulatory activity in the open field 15-30 min after the injection.

All rats received three injections. Two groups of rats were injected with AMPH only once, three weeks after the injection of CVP or oil alone. Three weeks before the

TABLE I

Groups and sequence of injections

Group	first injection	second injection	third injection
S/O/A, $n = 10$	Saline	Oil	AMPH
S/CVP/A, $n = 9$	Saline	CVP	AMPH
A/O/A, $n = 10$	AMPH	Oil	AMPH
A/CVP/A,$n = 10$	AMPH	CVP	AMPH

injection of CVP or oil, they were injected with physiological saline. Rats of the remaining two groups were injected with AMPH twice: three weeks before and three weeks after the injection of CVP or oil. It was expected that within-subject comparisons of the responses induced by AMPH before and after the CVP injection would enable more precise assesment of the pesticide effect. The groups and the sequence of injections within each group are given in Table I.

The "open field" used for testing rat locomotor activity was a square (100 x 100 cm) arena separated into 49 equal squares. The whole testing consisted of six sessions: three preinjection sessions and three postinjection ones. During each test session the rat was placed in the middle of the arena and observed for 10 min. The number of square border crossings (NBoC) was counted by a trained experimenter. After completing the preinjection testing, the rat was removed from the open field, injected on a nearby table, and then transferred to its home cage. In the case of AMPH or saline injections, the postinjection test session started 20 min after the injection. In case of CVP or oil injections it started 3 h after the injection, i.e when in the CVP injected rats the AChE inhibition should have reached maxium (Gralewicz and Soćko 1997).

The NBoC values were analyzed by a two-way repeated measures ANOVA (groups x sessions) and, in cases of a significant interaction effect, by one-way ANOVA and Tukeys test for pairwise comparisons (SPSS PC software, version 6).

The analysis performed on raw data revealed no significant differences in the preiniection NBoC values between groups nor between successive sessions within each group (sessions 1, 3 and 5). Therefore, individual postinjection scores were transformed into percent of respective preinjection ones and subjected to a two-way ANOVA (groups x injections). The effects of both main factors and the interaction appeared significant (effect of groups: $F_{3,35} = 13.53$, $P<0.0005$; effect of injections: $F_{2,34} = 85.07$, $P<0.005$; interaction: $F_{6,68} = 8.12$, $P<0.0005$. Consecutive within-group comparisons (one-way ANOVA followed by Tukeys test for multiple comparisons) revealed differences between the effects of successive injections in each group (group S/O/A ($F_{2,34} = 11.16$, $P<0.0005$); group S/CVP/A: ($F_{2,34} = 3.69$, $P<0.035$); group A/O/A: ($F_{2,34} = 43.22$, $P<0.0005$); group A/CVP/A: ($F_{2,34} = 58,65$, $P<0.0005$). In group S/O/A, the relative locomotor activity was significantly higher after the third injection than that noted after the first two injections which did not differ from each other. In the S/CVP/A group, the relative activities after the first (saline) and third (AMPH) injection showed no difference but both were significantly higher than the relative activity after the second (CVP) injection. We must emphasize, however, that the effect of AMPH in this group was highly variable; whereas in some rats locomotor activity after the injection was increased, in others no increase was seen. In the A/O/A and A/CVP/A groups, the activity after the second injection was significantly lower compared to that after the first and the third ones. In both these groups the relative activity after the third injection was significantly higher than that after the first one.

The comparisons between groups revealed significant differences after the first ($F_{3,35} = 14.11$, $P<0.0005$), second ($F_{3,35} = 10.35$, $P<0.005$), and third ($F_{3,35} = 5.59$, $P<0.003$) injections. After the first injection, the relative postinjection locomotor activity in groups A/O/A and A/CVP/A (given AMPH) was significantly higher than in groups S/O/A and S/CVP/A (given saline). Neither groups S/O/A and S/CVP/A nor groups A/O/A and A/CVP/A showed any difference. After the second injection the relative activities of groups S/CVP/A and A/CVP/A (given CVP) did not differ between themselves but were significantly lower than those in group S/O/A and A/O/A (given oil).

After the third injection (all groups given AMPH) the relative locomotor activity in group S/CVP/A was significantly lower compared to that in the remaining groups. In the S/O/A group, it was lower than in the A/O/A (the difference reached the significance limit) but it did not differ from that in the A/CVP/A group. Groups A/O/A and A/CVP/A did not differ between themselves (Fig 1).

It appears from the obtained results that i.p. administration of CVP or AMPH, each at a dose of 1.0 mg/kg, brings about opposite acute effects in rat behavior; CVP induces a decrease, and AMPH an increase in open-field locomotion. Both these effects are fully concordant with the literature concerning the acute behavioral effects of anticholinesterases (Pope et al. 1992, Ehrlich et al. 1993, Moser 1995, Richardson 1995) and AMPH (Lanier and Isaacson 1977, Fink and Smith 1979, Robinson and Becker 1986). The most important observations in the present study are: (1) the significant attenuation of the acute behavioral response to AMPH in rats with past acute exposure to CVP; (2) an apparent protection against this effect by a single 1.0 mg/kg dose of AMPH given three weeks before the exposure to CVP.

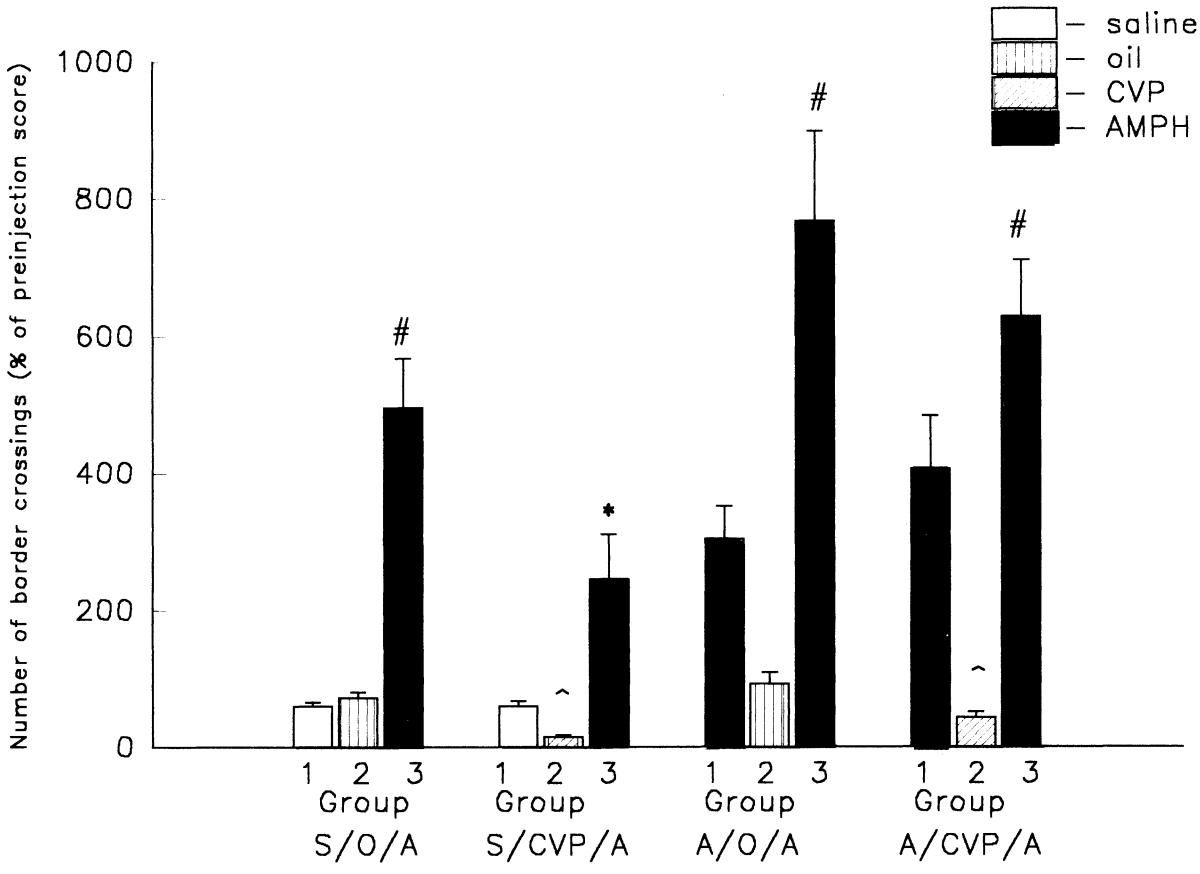

Fig. 1. Effects of acute CVP exposure (1.0 mg/kg, i.p.) on the locomotor stimulating effects of AMPH (1.0 mg/kg, i.p.) in rats pretreated with AMPH before the CVP exposure. The numerals 1 – 3 denote successive injections. The between-injection interval was three weeks. The bars denote group means (± SEM) of the postinjection open-field locomotor activity expressed as a percent of the preinjection values.#, $P<0.05$ compared to injection in a given group; *, $P<0.05$ compared to injection 3 in the remaining groups; ^, $P<0.05$ compared to injection 2 in groups S/O/A and A/O/A.

According to the results of our previous studies (Gralewicz and Soćko 1997), after a single exposure to a 1.0 mg/kg dose of CVP, blood and brain AChE activity returns to preexposure level within less than two weeks. Thus, a weaker behavioral response to AMPH in group S/CVP/A could not be ascribed to lowered AChE activity at the time of AMPH administration but rather to some long-term neuroadaptive changes, possibly resulting from a lowered activity of this enzyme in the past. As suggested by Duffy et al. (1979) on the basis of clinical observations, exposure to OP might result in a persistent increase in activity (or reactivity) of the central cholinergic system. An augmented cholinergic reactivity was also suggested by the character of the behavioral and EEG changes in rats tested several weeks after exposure to CVP (Gralewicz Soćko 1997). According to Overstreet et al. (1996), rats with inborn cholinergic hyperreactivity are characterized by a diminished behavioral responsiveness to dopaminergic agonists. In the present study, diminished behavioral responsiveness to AMPH, an indirect dopaminergic agonist, apparently characterized the rats of the S/CVP/A group. In these rats, however, this trait was acquired as a consequence of the previous exposure to CVP. Thus, the results of the S/CVP/A group might be regarded as an argument in support of a persistent increase in cholinergic activity following exposure to OPs. There remains the question of the primary locus of the enduring changes responsible for the behavioral hyposensitivity to AMPH after the CVP exposure. The antagonism between the cholinergic and dopaminergic systems in the control of psychomotor activation (Friedhoff and Alpert 1973, Dilsaver 1986) precludes a simple, straighforward answer to this question on the basis of any behavioral study. Both, the cho-

linergic and the dopaminergic systems are involved in mediation of the acute effects of OP exposure (Coudray--Lucas et al. 1987, Gotoh and Smythe 1992) and the long-term neuroadaptive changes following exposure are likely to occur in both these systems. A decreased responsivenes to AMPH challenge subsequent to OP exposure may thus be due to an increased cholinergic tone resulting from an increased sensitivity of cholinergic receptors as suggested by Duffy et al. (1979). Alternatively, it may result from a reduced functional efficiency of the dopaminergic neurons in some brain areas. Although a resolution of this problem must await further studies, the obtained results provide further evidence of some long-term alterations in the brain functional state following an OP exposure.

A significantly increased locomotor response to the second AMPH administration (third injection) in group A/O/A suggests sensitization, one of the well known persisting effects of exposure to psychostimulants (Kalivas and Stewart 1991, see also Robinson and Becker 1986 for a review). A comparison of the results in groups A/O/A and A/CVP/A shows that exposure to CVP did not hamper the development of this process; the behavioral response to the second AMPH injection was increased in both groups. However, the acute effect of CVP in group A/CVP/A was similar to that in group S/CVP/A. Taken together, this suggests that the long--term functional changes developing within the CNS as a result of an AMPH injection may prevent the development of the persisting effects of subsequent CVP exposure but do not protect the rat against the acute behavioral effects of the pesticide.

To sum up, the results of the present work show that in the rat, the behavioral sensitivity to AMPH given systemically decreases as a result of an acute CVP exposure and that this effect is detectable after a time long enough for the restitution of normal AChE activity. Therefore, they support the supposition that exposure to OP compounds and possibly other AChE inhibitors may result in persistent functional changes within the central nervous system.

Coudray-Lucas C., Le Guen A., Prioux-Guyonneau M., Cohen Y., Wepierre J. (1987) Changes in brain monoamine content and metabolism induced by paraoxon and soman intoxication. Effect of atropine. Xenobiotica 17: 1131-1138.

Darracq L., Blanc G., Glowinski J., Tassin J.P. (1998) Importance of the noradrenaline-dopamine coupling in the locomotor activating effects of D-amphetamine. J. Neurosci. 18: 2729-2739.

Dilsaver S. (1986) Cholinergic mechanisms in depression. Brain Res. Rev. 11: 285-316.

Duffy F.H., Burchfiel J.L., Bartels P.H., Gaon M., Sim V.M. (1979) Long-term effects of an organophosphate upon the human electroencephalogram. Toxicol. Appl. Pharmacol. 47: 161-176.

Ehrlich M., Shell L., Rozum M., Jortner B.S. (1993) Short--term clinical and neuropathologic effects of cholinesterase inhibition in rats. J. Am. Coll. Toxicol. 12: 55-67.

Fink J.S., Smith G.P. (1979) Abnormal pattern of amphetamine locomotion after 6-OHDA lesions of anteromedial caudate. Pharmacol. Biochem Behav. 11: 23-30.

Friedhoff A.J., Alpert J.(1973) A dopaminergic-cholinergic mechanism in the production of psychotic symptoms. Biol. Psychiatr. 6: 165-169.

Gershon S, Shaw F.H. (1961) Psychiatric sequelae of chronic exposure to organophosphorus insecticides. Lancet 1: 1371-1374.

Gotoh M., Smythe G.A. (1992) Effects of intracerebroventricularly administered neostigmine on hypothalamic monoaminergic neuronal activities in awake rats. Brain Res. 586: 340-343.

Gralewicz S., Soćko R. (1997) Persisting behavioral and electroencephalographic effects of exposure to chlorphenviphos, an organophosphorous pesticide, in laboratory animals. Int. J. Occup. Med. Environ. Health 10: 375-394.

Jackson D.M., Westlind-Danielsson A. (1994) Dopamine receptors: molecular biology, biochemistry and behavioural aspects. Pharmac. Ther. 64: 291-369.

Jones S.R., Gainetdinov R.R., Wigtman R.M., Caron M.G. (1998) Mechanism of amphetamine action revealed in mice lacking the dopamine transporter. J. Neurosci. 18: 1979-1986.

Kalivas P.W., Stewart J. (1991) Dopaminergic transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res. Rev. 16: 223-244.

Lanier L.P., Isaacson R.L. (1977) Early developmental changes in the locomotor response to amphetamine and their relation to hippocampal function. Brain Res.126: 567-575.

Metcalf D.R., Holmes J.H. (1969) EEG, psychological, and neurological alterations in humans with organophosphorus exposure. Ann. NY Acad. Sci. 160: 357-365.

Moser V. (1995) Comparison of the acute effects of cholinesterase inhibitors using a neurobehavioral screening battery in rats. Neurotoxicol. Teratol. 17: 617-625.

Overstreet D, H., Miller C.S., Janowsky D.S., Russel R.W. (1996) Potential animal model of multiple chemical sensitivity with cholinergic supersensitivity. Toxicology 111: 119-134.

Pope C.N., Chakraborti T.K., Chapman M.L, Farrar J.D. (1992) Long-term neurochemical and behavioral effects

induced by acute chlorpyrifos treatment. Pharmacol. Biochem. Behav. 42: 251-256.

Richardson R.J. (1995) Assessment of the neurotoxic potential of chlorpyriphos relative to other organophosphorus compounds: a critical review of the literature. J. Toxicol. Environ. Health 44: 135-165.

Robinson T.E., Becker J.B. (1986) Enduring changes in brain and behaviour produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res. Rev. 11:157-189.

Rosenstock L., Keifer M., Daniel W.E., McConnell R., Claypoole K. (1991) Chronic central nervous system effects of acute organophosphate pesticide intoxication. Lancet 338:223-227.

Savage E.P., Keefe T.J., Mounce L.M., Heaton R.K., Lewis J.A., Burcar P.J. (1988) Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch. Environ. Health 43: 38-45.

Yokoyama K., Araki S., Murata K., Nishikitani M., Okumara T., Ishimatsu S., Takasu, N. (1998) Chronic neurobehavioral and central autonomic nervous system effects of Tokyo subway station poisoning. J. Physiol (Paris) 92: 317-323.

Received 6 August 1999, accepted 10 February 2000