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

Sławomir Gralewicz,¹ Piotr Lutz¹ and Wiesław Szymczak²

¹Department of Toxicology and Carcinogenesis and ²Department 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 LD₅₀). 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.

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 increased 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 LD50). 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 |
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| Groups and sequence of injections | 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 assessment of the pesticide effect. The groups and the sequence of injections within each group are given in Table 1.

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 case of AMPH or saline injections, the rat was placed back on a nearby table, and then transferred to its home cage. 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 rat was removed from the open field, injected on a nearby table, and then transferred to its home cage. After the third injection (all groups given AMPH) the relative locomotor activity in group S/O/A was significantly lower compared to that in the remaining groups. 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.
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, straightforward 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, Gotth and Smythe 1992) and the long-term neuroadptive changes following exposure are likely to occur in both these systems. A decreased responsiveness 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 systematically 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.


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