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EFFECTS OF A SINGLE EXPOSURE TO CHLORPHENVINPHOS, AN ORGANOPHOSPHATE INSECTICIDE, ON HOT-PLATE BEHAVIOUR IN RATS

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Abstract. Effect of a single i.p. exposure to an organophosphate insecticide, chlorphenvinphos (CVP), in doses of 1.0 and 3.0 mg/kg (one third and one tenth LD_{50}, respectively), on the latency of the paw-lick response (hot plate test) was investigated in rats before and after a short inescapable footshock. The test was repeated twice on the 18th and 19th days after the exposure, i.e. after a time sufficient for a full recovery of cholinesterase activity in the blood and brain. On the first day of testing the groups did not differ with respect to the paw-lick latency before footshock. However, the paw-lick latency after footshock (the index of stress-induced analgesia) was significantly longer in rats exposed to the higher dose of CVP (3.0 mg/kg) than in the control animals. Twenty four hours later, in the control animals, the paw-lick latencies before footshock were shortened in comparison with those recorded on the day before. An opposite effect was observed in the rats exposed to 3.0 mg/kg of CVP. The data suggest that some alterations in the brain functional state may outlast the CVP induced depression of cholinesterase activity.

INTRODUCTION

Organopohosphate (OP) compounds — irreversible inhibitors of cholinesterase (ChE) — are widely used as pesticides. Reports suggesting that even a single exposure to OPs may result in alteration of brain function outlasting the period of the ChE recovery are growing in number. Psychological dysfunctions, including memory and emotional disturbances, were detected in persons with a history of OP exposure in the past (13, 15). EEG abnormalities have been found in humans (6) as well as in primates (2) almost a year after repetitive or even single

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exposure to sarin (isopropyl methylphosphonofluoridate). It has been suggested that these changes may consist of persistant effects of past cholinergic hyperactivity (6). The human reports, however, are frequently inconclusive and need confirmation by animal studies. Unfortunately, most of the existing behavioral data concerning effects of OP exposure on laboratory animals refer to the period of lowered ChE activity (9, 12).

The experiment described below concerns chlorphenvinphos (phosphoric acid, 2-chloro-1/2,4 dichlorophenly/vinyl diethyl ester — CVP), an OP pesticide. An attempt was made find out the effect of a single exposure to CVP on the level of analgesia resulting from a short inescapable footshock (1). A hot-plate test was used to measure the level of analgesia. According to some authors the occurrence of analgesia in such a situation is contingent upon a test for analgesia before footshock, and as such is analogous to passive avoidance learning (8), i.e. a form of learning motivated by fear. The documented involvement of the cholinergic system in the control of negative emotions (3, 5) and memory (4, 7) on the one hand, and the reports suggesting memory disorders and emotional disturbances in humans after OP exposure on the other, justified the use of the above test in the present studies. The interval between exposure and the start of testing was 18 days. According to our previous data (16) it was sufficient for full ChE recovery in the blood as well as in the brain.

MATERIALS AND METHODS

Animals. Male Wistar rats 100—120 days old and of 250—300 g of body weight at the beginning of the experiment were used.

Apparatus. The equipment consisted of a hot-plate and a shock chamber. The hot-plate was a copper box (350×350×35 mm) filled with water. The water temperature was kept constant at 54.5°C (±0.2). A plastic translucent cylinder (280 mm diameter 250 mm height) with a top cover formed an enclosure preventing the rat from stepping off the plate. The shock chamber was a tight (100×200×400 mm) equipped with a metal grid floor which could be electrified.

Pesticide and exposure condition. CVP (technical grade) was supplied by the manufacturer (ORGANIKA-AZOT, Jaworzno, Poland). Before use, it was dissolved in warm (39—40°C) sterile olive oil. The effect of two doses of CVP were tested: 3.0 mg/kg and 1.0 mg/kg, i.e. about 1/3 and 1/10 of the rat LD_{50}, respectively (10). The rats used for testing the 3.0 mg/kg dose were denoted as the High Dose Group (H) and those

for testing the 1.0 mg/kg dose as the Low Dose Group (L). Animals from each group were assigned randomly to two subgroups: the exposed (E) and the control (C). Thus, group H and group L each consisted of two subgroups: HE and HC and LE and LC, respectively. The exposed animals were given CVP intraperitoneally in volume of 0.7—1.0 ml. The control animals were given pure oil in a similar volume.

Testing procedure. The test was performed twice, i.e. on the 18th and 19th days after exposure. It consisted of placing the rat on the hot-plate within the plastic enclosure. After occurrence of the expected response — licking the foot — or after 60 seconds the animal was transferred into the shock chamber where it received trains of electric footshocks, one every five seconds, for two minutes. The train duration was one second, the frequency of electric pulses in the train — 5 Hz, pulse duration — 6.5 msec, and the current intensity — 3.5 mA. Immediately after shocking, the rat was transferred onto the hot plate again. The latency of the paw-lick response before shocking (L_1) and after (L_2) was carefully measured with a stop-watch. The stop-watch was switched on at the moment when the rat, being placed into the plastic enclosure, touched the hot plate with four paws. It was switched off immediately after the first lick at the foot of the right or left hind limb had been noticed.

Statistics. The obtained data were analysed with a parametric one-way ANOVA. Detailed comparisons were made with the use of the Sheffe test (17).

RESULTS

a) Changes in general behavior. Acute symptoms of intoxication were noted only in the HE subgroup. They appeared with a latency of 15—30 min and consisted of decreased motor activity, tremor and lacrimation. The vegegative symptoms disappeared within six hours after the exposure. Motor sluggishness was noted up to three days after exposure.

b) Hot-plate behavior. In order to avoid confusion, the paw-licking latencies before and after shocking on the 18th day were denoted as L_1 and L_2, and those recorded on the 19th day as L_1 and L_2 respectively. As no differences were found between HC and LC subgroups during a preliminary analysis, their data were pooled, and in the majority of comparisons the subgroups were treated as one C Group. The obtained data are presented in Table 1. On the 18th day the groups did not differ with respect to L_1. They differed, however, with respect to L_2 ($F_{2,41}=8.62$, $p < 0.01$), as well as with respect to the L_2/L_1 ratio ($F_{2,41}=8.12$, $p < 0.01$);

TABLE 1. Effect of an inescapable footshock on the latency of the paw-lick response (hot-plate behaviour) in rats on the 18th and 19th days after a single ip exposure to chlorphenvinphos

Group (CVP dose)	18th day after CVP exposure			19th day after CVP exposure			
	L_1'	L_2'	L_2'/L_1' (%)	L_1'' *	L_2'' **	L_2''/L_1'' (%) ***	L_1''/L_1' (%)
HE 3 mg/kg n = 13	14.4 ±4.7	38.6[a] ±15.7	269.9[b] ±84.4	25.6 ±19.3	37.1 ±12.5	180.0 ±68.3	175.4[c] ±94.7
LE 1 mg/kg n = 10	15.4 ±3.1	23.8 ±6.5	160.1 ±63.2	14.4 ±4.3	24.2 ±8.5	173.6 ±51.5	94.9 ±30.5
C control n = 18	13.6 ±3.8	26.4 ±8.9	204.5 ±62.8	10.3 ±6.9	17.7 ±6.1	199.5 ±101.4	81.7 ±33.7

legend:

L_1 and L_2 — paw-lick latency in seconds (Mean and SD) before and after the footshock, respectively

a — differs from LE ($p < 0.01$) and from C ($p < 0.05$)

b — differs from LE ($p < 0.01$) and from C ($p < 0.05$)

c — differs from LE ($p < 0.05$) and from C ($p < 0.05$)

* — Three rats did not respond within the adopted 60 sec time limit. 60 sec was the accepted L_1'' value for these animals during calculation of the mean.

** — Five rats did not respond within the accepted 60 sec time limit. 60 sec was the accepted L_2'' value for these animals during calculation of the mean.

*** — Data from rats which did not respond before or after the footshock were not taken into account during celculation of the mean.

in rats of the HE subgroup the L_2 was significantly longer and, consequently, the L_2/L_1 ratio significantly higher than in the remaining two groups. No significant differences between groups were found when L_1 and L_2 absolute values and the L_2/L_1 ratio were compared. It was due to high individual variability during the second trial. The paw-licking response failed to appear within the 60 second limit in three animals of the HE subgroup before shocking, and in five of the same group after shocking. Moreover, a closer examination of the data revealed that in control rats the L_1'' values were usually shorter than L_1' ones. An opposite relationship was noted in the exposed animals. Statistical comparisons of the L_1''/L_1' ratio revealed that the differences were significant ($F_{2,41}=11.35$, $p < 0.001$). In the HE subgroup, the L_1''/L_1' ratio was significantly higher than in the C and LE Group. LE subgroup did not differ from the group with respect to the L_1''/L_1' ratio.

DISCUSSION

According to literature data, paw-lick latency on a hot plate may remain increased for seven days after a single exposure to a sublethal dose of soman (9), and the jump response to a painful stimulus is reduced in the course of repetitive exposure to the same OP (14). These data suggest an OP-induced decrease in sensitivity to pain, an effect which may be related to a lowered ChE activity at the time of testing. The results of our experiments presented here suggest that 18 days after a single exposure to 3.0 mg/kg of CVP the pain sensitivity in the rat before footshock is normal, but the stress-induced analgesia is significantly higher. What is more, in the exposed animals, the effect of stress seems to dissipate more slowly which has been evidenced in the increased paw-lick latencies seen 24 hours after the stressful experience.

According to Gower and Tricklebank's findings (8), the analgesia produced by a short inescapable footshock is contingent upon the test for analgesia before the shock, and as such it may be regarded as a form of passive avoidance learning. They also have found that this form of analgesia is enhanced by cholinergic agonists and prevented by cholinergic (muscarinic) antagonists (8). On the basis of the above data, the longer L'_2 and the higher L''_1/L'_1 ratios in our CVP exposed animals may be regarded as evidence of improved memory or enhanced fear, on the one hand and an increased activity (or reactivity) of the cholinergic system, on the other. This increase, however, cannot be due to lowered ChE activity and an excess of acetylcholine in the cholinergic synapses at the time of testing; as our previous experiments have shown, after a single ip administration of CVP, ChE activity in the blood and the brain returns to a normal level within 14 days in the case of the 3.0 mg/kg dose and within four in the case of the 1.0 mg/kg dose (16). Therefore, other changes, concerning possibly the postsynaptic membranes, may be considered.

To sum up, the obtained results show that in rats some behavioural consequences of a single exposure to CVP may be detected after a time sufficient for ChE recovery and in this respect they are consistent with human (6, 13, 15) and primate (2) data.

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