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Comparison of the effect of subacute organophosphate exposure on the cortical and peripheral evoked activity in rats

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Abstract

Organophosphates are irreversible blockers of acetylcholinesterase and are widely used as insecticide agents. Their action is not limited to the target organisms so that occupational or food-borne exposure of humans usually leads to neurotoxicity in which several other mechanism, apart from cholinesterase inhibition, may play a role. In the present study, rats were treated with three different organophosphates (chlorfenvinphos, diisopropyl fluorophosphate, and dimethoate) for 4 weeks, and alterations in two forms of stimulus evoked activity—somatosensory and visual cortical sensory evoked potentials and peripheral nerve action potential—were compared. In the treated rats, there was significant increase in the somatosensory evoked response latency and non-significant increase in its duration. In the visual evoked potential, only duration was altered. The conduction velocity of the peripheral nerve was decreased. Comparison of the changes in the cortical- and peripheral evoked activity showed that the slowed peripheral impulse conduction only partly explains the increase in the cortical response latency. Hence, possible mechanisms of direct cortical action of the organophosphates are also discussed.

Keywords: Organophosphate; Cortical evoked potential; Nerve action potential; Rat

1. Introduction

Organophosphate (OP)[1] insecticide agents are multi-substituted derivatives of phosphoric acid [1]. Their primary mode of action as neurotoxic substances is irreversible inhibition of acetylcholinesterase (AChE) [2] through covalent acylation. OPs are considered—in spite of the time-dependent reactivation seen with many of them—irreversible AChE blockers. The toxicity of OPs is, however, not limited to the target organisms, so that they can represent a major ecotoxicological and hygienic toxicological hazard when released into the environment. The primary target of OPs in the

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[1] *Abbreviations used:* OP, organophosphate; ACh, acetylcholine; AChE, acetylcholinesterase; CVP, chlorfenvinphos; DFP, diisopropyl fluorophosphate; DIM, dimethoate.

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Table 1
Values of the measured parameters of cortical and peripheral evoked activity

Parameter (ms)	Control	CVP	DFP	DIM
SS EP latency	8.52 ± 0.99	$12.03 \pm 1.60^*$	$10.80 \pm 0.47^*$	$10.90 \pm 0.65^*$
SS EP duration	11.87 ± 1.55	12.91 ± 2.45	13.38 ± 1.74	11.28 ± 1.67
VIS EP latency	96.25 ± 6.95	98.12 ± 7.07	94.25 ± 2.87	94.75 ± 6.02
VIS EP duration	75.50 ± 3.11	81.23 ± 18.39	83.75 ± 9.18	$85.67 \pm 21.97^*$
Tail nerve rel. refr.	14.13 ± 4.85	11.91 ± 1.32	13.82 ± 5.99	13.18 ± 4.07
Tail nerve abs. refr.	1.66 ± 0.20	1.80 ± 0.27	1.76 ± 0.25	1.79 ± 0.22
Tail nerve cond. vel.	14.45 ± 0.81	$12.24 \pm 0.67^*$	$13.28 \pm 0.69^*$	$13.36 \pm 0.53^*$

All values are means $\pm$ SD; $n = 10$.
* $p < 0.05$.

human or animal organism is the nervous system; but the mechanisms of neurotoxicity are, in numerous cases, not yet well known. Acetylcholine (ACh) is in the periphery neurotransmitter. In the brain, however, it is mostly a regulator, e.g., of cortical basic activity [3] but also of sensory evoked responses in the cortex [4,5]. In our previous studies [6,7] subchronic (12 weeks) administration of different OPs (including dimethoate) to rats resulted in characteristic changes of the cortical sensory evoked potentials and the peripheral evoked activity. It was also found, however, that the effect of OPs was not influenced by atropine [8], and the effects on the cortical and the hippocampal activity (the latter known to have cholinergic modulation) [9] were found to be of opposite direction [10]. Moreover, the blockage of blood or brain cholinesterase was not necessarily in parallel with the changes seen on the nervous activity [11,12]. In many cases, the neurological effects in humans by far outlasted the period of AChE inhibition [13,14]. Following a single dose of sarin to monkeys, Duffy and Burchfiel [15] observed prolonged EEG alterations. These reports raised the possibility that increased cholinergic influence on the cortex is not, or is not the sole, explanation of the changes seen in the stimulus-evoked cortical activity on OP administration. One of the alternative explanations could be the altered impulse conduction between the sensory organ and the cortical focus. To see whether and to what extent the alteration of the axonal impulse conduction plays a role, a subacute experiment was carried out in rats treated with the OPs chlorfenvinphos (CVP) and dimethoate (DIM)—two insecticide agents [1], and diisopropyl fluorophosphate (DFP)—a laboratory standard OP. The two insecticides were chosen because both have practical agricultural application and because there is a marked difference in their affinity to brain acetylcholinesterase. (The latter is to be investigated in further studies, based on [16,17].) In this paper, the effect of the OPs on stimulus-evoked peripheral and cortical activity will be analyzed (see Table 1).

2. Materials and methods

Male Fisher F344 rats, weighing 220–250 g at the beginning, were used. The animals were housed under controlled conditions of temperature (22–24 °C) and photoperiod (12-h light/dark cycle with light starting at 6:00 a.m.), with free access to standard food and drinking water. Groups of 10 animals were treated with 1/25 LD_{50} doses of the OPs (CVP, 1.2; DIM, 18; and DFP, 0.2 mg/kg b.w.) dissolved in distilled water, administered per os by gavage, in a five times per week schedule for 4 weeks. The treatment solution had a specific volume of 1 ml/kg b.w., and was prepared by 100 $\times$ dilution from a stock solution immediately before use. The stock solutions were made up in 96% ethanol and kept in fridge at 4 °C. Control rats received distilled water. LD_{50} was previously determined in animals of the same strain.

At the end of the treatment period, cortical and peripheral evoked activity elicited by various stimuli were recorded in an acute preparation. On the first post-exposure day, the animals were anesthetized with 1000 mg/kg urethane ip. [18]. The head was fixed in a stereotaxic frame, the left hemisphere was exposed by opening the skull, and

ball-tipped silver wire electrodes were placed on the primary somatosensory and visual cortical areas. Somatosensory stimulation was done by a pair of needles delivering weak electric shocks to the whiskery part of the skin (rectangular stimuli, 3–4 V, 0.05 ms—just supramaximal). Visual stimulation was performed by flashes (ca. 60 lx) delivered from a flashbulb unit via an optical fiber directly into the contralateral eye of the rat. One train of 50 stimuli of each modality, with 1 Hz repetition frequency, was applied to the rats and the evoked activity was recorded. Impulse conduction in the tail nerve was examined by means of a stimulating (similar electric stimuli as for the whiskers) electrode pair inserted at the base of the tail and a recording electrode pair placed at 50 mm more distally [19]. All recording of the evoked activity, and subsequent off-line measurements, were performed by a PC using the NEUROSYS 1.11 software (Experimetria, UK). The evoked responses were averaged. On the cortical responses, latency and duration of the main waves was measured. Latency was defined as the time elapsed between the stimulus (arrow in Fig. 1) and the onset of the cortical response (L in Fig. 1), and duration as the interval between the onset and end of the response (i.e., between L and D in Fig. 1). On the tail nerve response, only latency (L_1 and L_2) was measured. Conduction velocity was determined by dividing the distance between the stimulating and recording electrode pair by the latency measured. For the refractory periods, double-pulse stimulation was performed, the latency of the first and second nerve action potential measured (see Fig. 1), and the second:first ratio calculated. By plotting the latency ratio against the inter-stimulus time, a hyperbolic curve was obtained, the horizontal and vertical asymptotes of which correspond to the relative (latency of the second response longer than that of the first) and absolute (latency of the second response infinitely long) refractory period, respectively. The exact values were determined by linear transformation of the curve [20]. Relative changes (treated/control) of the parameters studied were calculated on the basis of group averages. To see if the changes were significant, two-sample t test (treated group vs control group) was used after normality check using the Kolmogorov test.

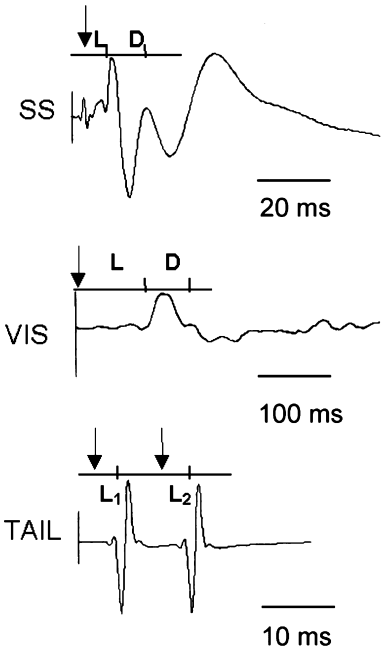

Fig. 1. Sample curves of the cortical (SS, somatosensory; VIS, visual) evoked response and the peripheral (TAIL) nerve action potential, and measurement of latency (L) and duration (D). Arrow, stimulus. Latency is the time between the stimulus and the onset of the response, and duration is the interval between the onset and end of the response, both indicated on the timelines above the corresponding curves. Determination of the refractory periods of the tail was based on the latencies L_1 and L_2.

3. Results

During the treatment period and up to the time of preparation, no major signs of general toxicity (such as abnormal locomotion, muscle fibrillation, salivation) were observed. The body weight gain of the animals was normal.

Each of the organophosphates used had an effect on both the cortical and peripheral evoked activity. On the somatosensory cortical evoked potential, increase of the onset latency was the most important effect. As seen in Fig. 2A (showing relative changes, see Table 1 for measured absolute values), all three OPs caused an over 20% increase in the latency ($p < 0.05$; $n = 10$). CVP was the most effective. The duration of the somatosensory evoked potentials showed a slight, non-significant lengthening on application of each OP. The

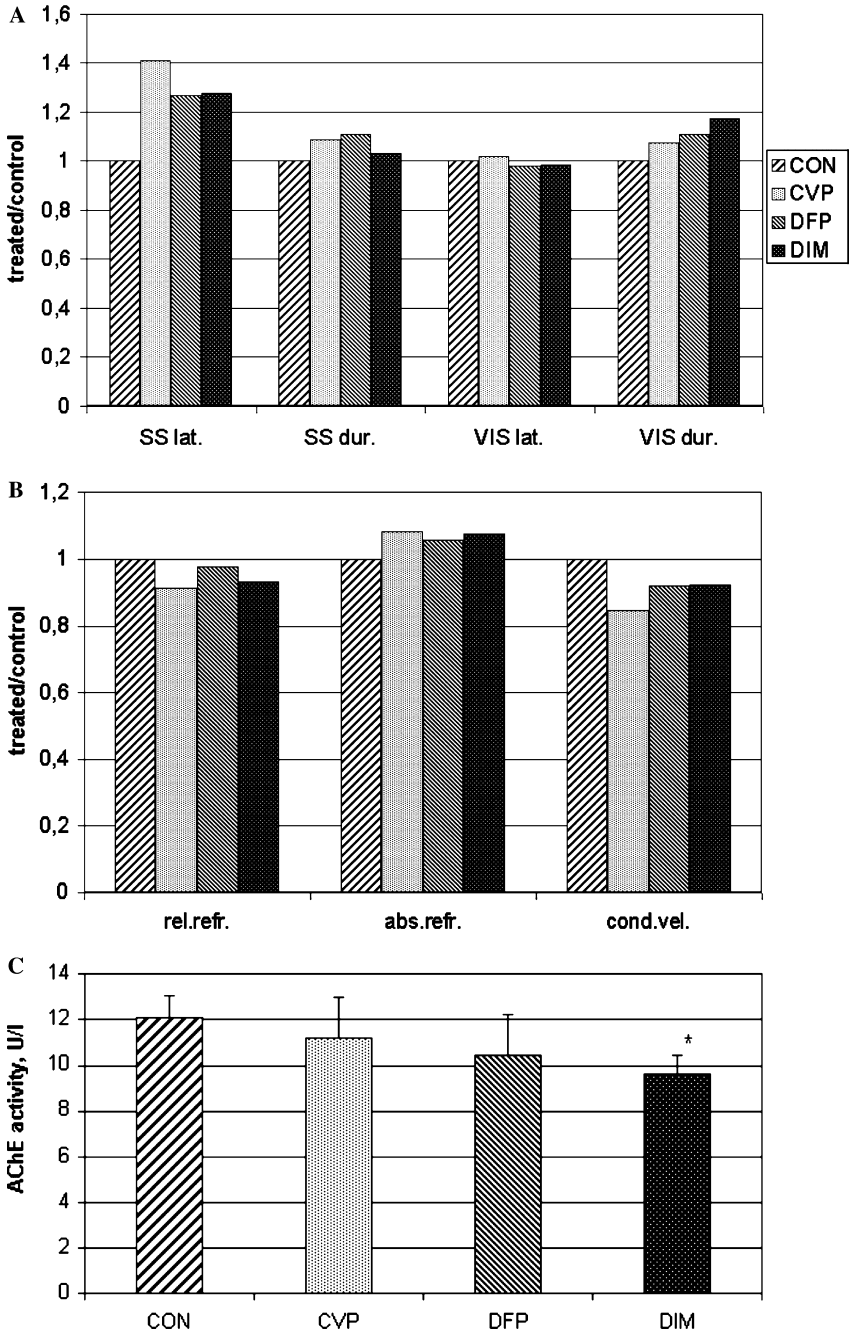

Fig. 2. (A and B) The effect of the organophosphates on latency and duration of the sensory evoked potentials (A) and on the refractory period and conduction velocity of the peripheral nerve impulse (B), given as relative alteration (shown are group mean values, normalized to control; $n = 10$). SS, somatosensory; VIS, visual; lat., latency; dur., duration; rel.refr., relative refractory period; abs.refr., absolute refractory period; and cond.vel., impulse conduction velocity. (C) Acetylcholinesterase activity in the whole brain (means $\pm$ SD; $n = 10$; $^*p < 0.05$ vs control). Treatment groups: see box in (A).

alterations of the visual evoked potential were less clear-cut (mostly non-significant). There was no noticeable change in the latency. The lengthening of the duration was significant ($p < 0.05$; $n = 10$) only with DIM which had the strongest effect on this form of activity.

In case of the tail nerve action potential (Fig. 2B), conduction velocity (calculated on the basis of the response latency, see Section 2) was the parameter most affected by the OPs ($p < 0.05$; $n = 10$ for all three substances). Again, the velocity was most strongly reduced by CVP. The effect on the refractory periods of the nerve was non-significant.

In separate groups of rats, receiving the above oral treatment for 12 weeks, brain acetylcholinesterase activity was determined (along with a number of other biochemical and electrophysiological measurements not dealt with here). As seen in Fig. 2C, the changes in the enzyme activity caused by the three OPs were not congruent with the changes in the physiological parameters shown in Figs. 2A and B.

4. Discussion

Increased latency of the somatosensory cortical evoked potential and slowed conduction velocity of the peripheral nerve, the two major effects of OPs observed in the present work, seem to be in line. Similar effects were observed in our previous works with partly different OPs (dimethoate, dichlorvos, methyl parathion) and dosing times (up to 12 weeks) [6,7,21]. Reduced conduction velocity may well have caused the delay of the cortical somatosensory response (but probably not of the visual one where no peripheral nerves are involved) and there are indications that exposure to OPs results in functional damage to peripheral nerves in humans [22,23] and animals [24]. In the latter, effect on AChE was supposed as mechanism. On the other hand, the relative latency increase of the cortical evoked potential was more intense than the relative slow-down of the peripheral nerve action potential. In Fig. 3, the correlation of the cortical response latency and peripheral conduction velocity is given. The slope

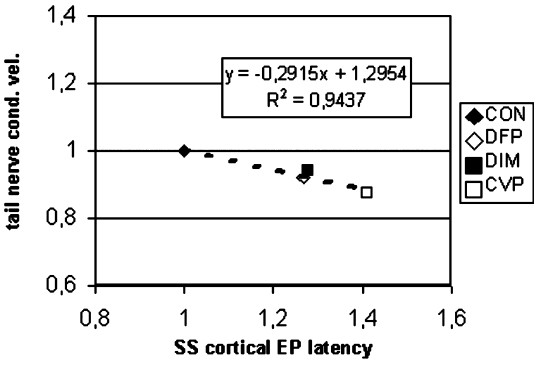

Fig. 3. Correlation diagram showing the relationship of the somatosensory cortical evoked response latency (abscissa) and the tail nerve conduction velocity (ordinate) in control and treated rats. Group mean values of both parameters were normalized to control (i.e., control = 1) and plotted. Treatment groups: see box. (Inset) Equation of the fitted line (to show the slope parameter and the correlation coefficient).

parameter (see inset) shows that the slowed peripheral impulse conduction can be responsible only for ca. 1/3 of the cortical latency increase. It was thus likely that decreased impulse conduction was merely one of the factors responsible.

Another effect involved can be the direct influence of OPs on the central processes. Visual cortical-evoked potential latency was significantly prolonged in humans after acute sarin exposure [25]. It was found, however, that this effect was not correlated to AChE inhibition. In animals, some authors reported no correlation between effects of OPs on AChE and on various functional outcomes [26]. In other works, the effect was apparently cholinergic, as in the depression of the visual evoked potential described in sarin-treated cats [27]. In rats, cortical somatosensory responses have cholinergic modulation [4] which is an obvious site of action for OPs. This would be a possible explanation of the effects seen in our work but, as shown by Fig. 2C, the changes of the evoked cortical and peripheral activity and of AChE activity were not is parallel.

Besides the above, certain OPs, including DFP, exert a direct action on, e.g., cortical muscarinic [28] and $GABA_A$ [29] receptors. The former effect could be another way how OPs affect cholinergic modulation of cortical activity, whereas the latter can, by decreasing recurrent inhibition, blur the

normally quite sharp boundaries of cortical excitation, both in space and time (response duration).

Several, central and peripheral, mechanisms can thus be involved in the effect of OP exposure on sensory performance. These all need to be further studied, and be taken into consideration in the neurological evaluation of exposed individuals. Studies of this kind may also help in choosing optimal functional indicators of OP exposure.

Acknowledgments

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