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Research report

OPPOSITE SHORT-TERM CHANGES INDUCED BY AN ORGANOPHOSPHATE IN CORTICAL AND HIPPOCAMPAL EVOKED ACTIVITY

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Summary: Organophosphates are the most widely used pesticides throughout the world. The considerable amount brought out to the environment poses a risk on the whole population. As organophosphates are neurotoxic substances and their residues can persist in the environment for several weeks, their influence on the nervous system of humans and animals is of principal interest. In the present study, we investigated the alterations induced by dichlorvos, a common pesticide substance, in parameters of somatosensory evoked potentials and hippocampal evoked population spikes of rats. The changes of the cortical vs. hippocampal evoked responses were opposite and only hippocampal effects could be directly explained through an increased cholinergic activity.
Keywords: organophosphate, cholinergic system, cortical evoked potential, hippocampal population spike, rat

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INTRODUCTION

Organophosphates (OPs), multi-substituted organic esters of phosphoric acid (WHO, Geneva, 1986), are well known synthetic neurotoxic substances and are primarily used as insecticides. Their main mode of action is an irreversible inhibition of the acetylcholinesterase enzyme (AChE). As, however, the OP's toxicity is not confined to insects or arthropods, using them poses a risk to other living species including man. Most of the early reports on OP toxicity, first of all in human cases, dealt mainly with changes in the acetylcholine (ACh) metabolism (see Kaloyanova's review, 1975). Metcalf and Holmes (1969) described prolonged alterations of the central nervous system function in humans after OP exposure. Changes in the EEG were later confirmed (Duffy et al., 1979).

Dichlorvos (DDVP) is a highly effective and toxic OP (WHO, Geneva, 1989). Due to its anticholinesterase potency, it induces an accumulation of ACh in the brain (Stavinoha et al., 1976). The changes of cortical electrical activity caused by dichlorvos (and related OPs) are, however, not always accompanied by corresponding changes in ACh levels or AChE activity (Dési, 1983) or they outlast them (Gralewicz et al., 1991).

It has been reported by the same authors (Gralewicz et al., 1989) that, parallel with the OP-induced changes in EEG (described also in Nagymajtényi et al., 1988 and Dési et al., 1994), a characteristic increase in hippocampal theta activity, possibly due to an increased cholinergic input from the septal area (Szerb et al. 1977; Wainer et al., 1985) can be seen; even in (e.g. urethane) anaesthesia (Monmaur et al., 1993). This, considering the central role of hippocampus in memory and behavioral functions, may give some explanation for OP-induced changes of those (Schulz et al., 1990). Previous research of our laboratory (Dési et al., 1991; Nagymajtényi et al., 1994) has shown that, beside EEG changes, cortical evoked potentials also undergo characteristic changes on OP treatment.

In the present experiments, our aim was to compare the effects of acute dichlorvos treatment on the typical forms of cortical vs. hippocampal evoked activity i.e. the somatosensory evoked potential (Schlag, 1973) and the hippocampal population spike (Andersen et al., 1971). We wanted to see whether there is any correlation between the dichlorvos-induced alterations of the two and whether these can be explained by the AChE inhibition.

METHODS

The experiments were done on male Wistar rats of about 300 g body weight. Following urethane anaesthesia (1000 mg/kg b.w., i.p.) the animal's head was fixed in a stereotaxic frame and the skull above the left hemisphere was opened. Wounds were sprayed with lidocaine. Following surgery the animal, with its body covered in a warm cloth, was put aside for at least 30 min for recovery. Somatosensory cortical evoked potential was recorded over the barrel field with a silver surface electrode. Electrical stimuli were delivered to the contralateral whiskery skin by a pair of needles. For recording hippocampal activity, a bipolar stimulating electrode was positioned to the perforant path (AP: 6, L: 4.5, V: 4 mm stereotaxic coordinates) while the recording glass microelectrode was put in the CA1 region (AP: -3.5, L: 2.5, V: 2 - 4 mm (taking bregma as reference point, according to the rat brain atlas of Paxinos and Watson, 1982). Parameters for electrical skin stimulation were 3 V/ 0.2 ms, 1 Hz; for perforant path stimulation: 1 mA, 0.5 ms, 0.1 Hz. Low frequency hippocampal stimulation was chosen to avoid any facilitation. Fifty evoked responses were averaged using the "pCLAMP" (Axon Instruments, Inc, Foster city, CA, USA) computer software. Latency, duration and amplitude of the evoked responses were measured later, off line, using the same software. Relative changes and principal statistical parameters of those for the whole group (i.e., 6 animals each) were calculated. In case of evoked potentials, start latency (from the stimulus artifact), total duration and peak-to-peak amplitude was measured between specific points as shown in Fig. 1A. For population spikes, the measurements were similar.

After taking 3 - 5 control records (one in 30 minutes), 1/5 LD_{50} (16 mg/kg b.w.) DDVP was applied per os by gavage, via a plastic tube lead through the oesophagus into the stomach during preparation.

Per os LD_{50} for DDVP was 80 mg/kg b.w. as determined in preliminary experiments (Dési et al., 1991). Records were taken again every 30 minutes up to 120 minutes from administration. This experimental sequence meant that every animal served as its own control. Beyond that, a saline-treated control group was recorded for 2.5 hours to prove that there were no spontaneous fluctuations interfering with the measurement.

Acetylcholinesterase activity in the brain was determined in separate groups of animals (also anaesthetized), one group receiving the same OP treatment as above and another serving as control. The method of Ellman et al. (1961) was used.

RESULTS

Cortical evoked potential

Somatosensory cortical evoked potentials had a clear biphasic shape which was sometimes distorted by the stimulus artifact (Fig. 1A). Punctum maximum of the response was searched by moving the recording electrode on the cortical surface. DDVP administration induced a steady increase in the latency and duration of the evoked responses. In a typical experiment, the latency changed from 2.91 ms (control) to 4.05 ms (at 120 min) and its duration from 2.55 ms (control) to 3.14 ms (120 min). Amplitude of the response, however, was diminished, e.g. from 2126 μV (control) to 1525 μV (120 min). Time courses of the relative changes of the evoked response are demonstrated in Fig. 2. Averaged changes in the latency, duration and amplitude of the evoked responses after treatment and in controls are given in Table 1.

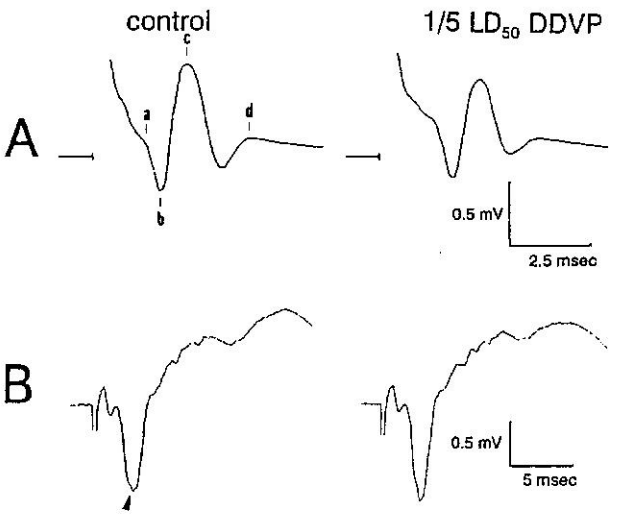

Fig. 1. Samples of somatosensory evoked potentials (A; average of 50) and hippocampal population spikes (B: average of 50). Left: control, right DDVP treated (60 and 65 min, respectively). Upward deflection means positivity. Note different calibration for A and B. Measurements on the evoked potential (A): latency, between stimulus artifact and a; duration, between a and d; amplitude, between b and c. A solid arrow points to the peak of the population spike (B), latency and amplitude of which was measured between the stimulus artifact and the peak

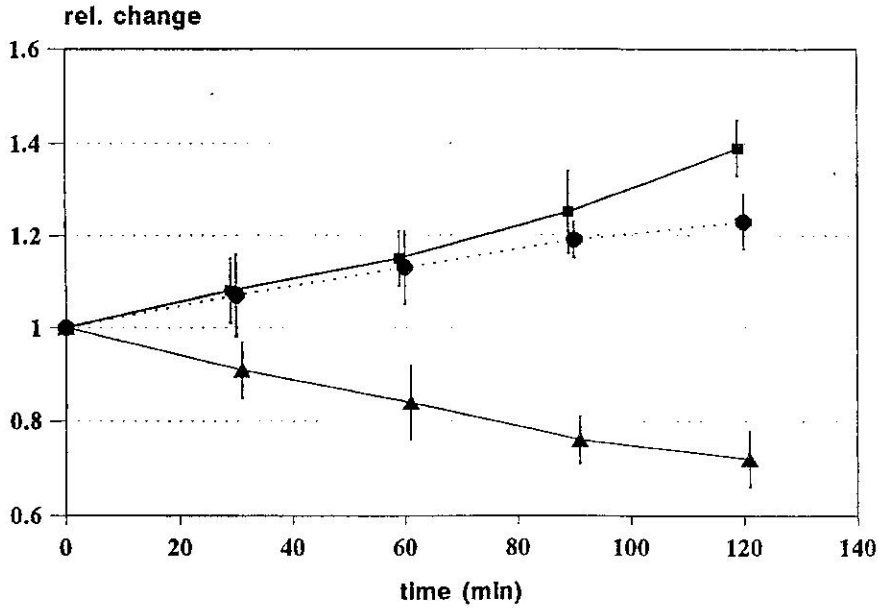

Fig. 2. Time course of the relative changes in parameters (squares: latency, circles: duration, triangles: amplitude) of the somatosensory evoked potential. DDVP was given (per os) at 0 min

Hippocampal population spike

This type of evoked activity can be described as a brief negative surge superposed on the field potential (Fig. lB). It is a result of the synchronous discharge of the pyramidal neurons. In some of our experiments, we saw the population spike in an inverted form, but, based on shape and latency, its identity was undoubted.

The changes in the population spike caused by DDVP treatment were opposite to those seen on cortical evoked potentials and the features most affected were also dissimilar. There was no significant alteration in latency and duration of the population spikes. Their amplitude, however,- increased, e.g. from 810 μV (control) to 1130 μV (at 120 min). Figure 3 shows the time course of the changes in the population spikes' parameters. Averaged relative changes are given in Table 1.

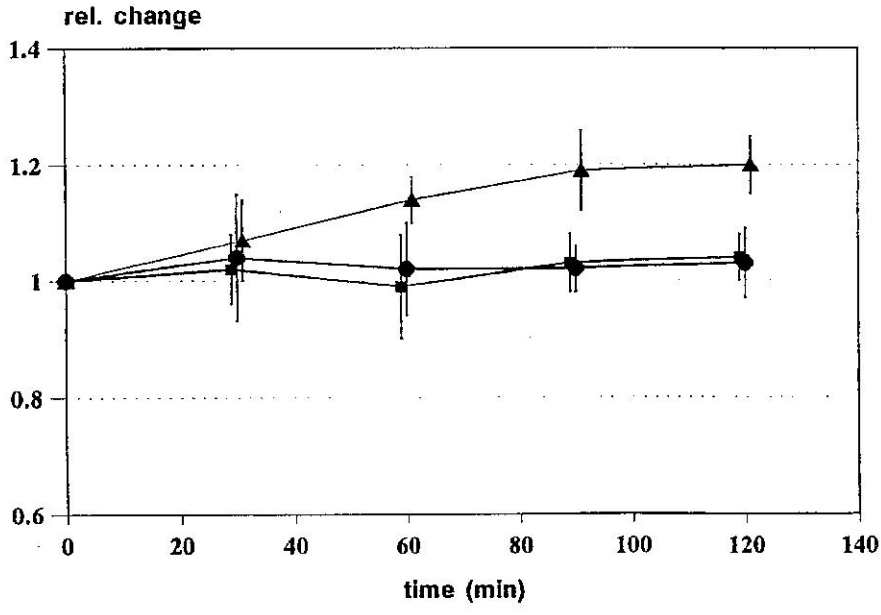

Fig. 3. Time course of the relative changes in parameters (squares: latency, circles: duration, triangles: amplitude) of population spikes. DDVP was given (per os) at 0 min

Acetylcholinesterase activity

In an untreated group (n=6) of animals, averaged (mean ± s.d.) specific AChE activity was 0.039 ± 0.003 mg substrate/μg protein × minute. In the DDVP-treated group it was 0.033 ± 0.004, resulting in a relative activity of 85%.

Table 1. Averaged (mean ± s.d., n=6) final relative changes (in % of control) of the parameters of the somatosensory evoked potential and the hippocampal population spike in the DDVP-treated and the control (saline-treated) groups.
Changes marked with * were significant ($p < 0.05$) using the paired t-test.

		Amplitude	Latency	Duration
Somatosensory evoked potential	DDVP	72.6*±4.4	139.2*±5.4	123.1*±3.7
	control	97.5±5.2	103.1±4.3	102.5±4.4
Hippocampal population spike	DDVP	120.4*+3.1	104.2±5.7	102.8±6.1
	control	101.6±6.2	95,4±3,9	103.1±4.4

DISCUSSION

Organophosphate-induced changes in the brain electrical activity have been well known. Most observations dealt with the spontaneous activity - first of all that of the cortex (Duffy et al., 1979; Duffy and Burchfiel, 1980; Philippens et al., 1992) and in lesser extent of the hippocampus (Gralewicz et al., 1989, 1991). Evoked activity - as shown above - also undergoes certain alterations on organophosphate action. The changes of evoked cortical activity, seen in the present experiments, are in concordance with those found in earlier works of our group (Dési et al., 1991; Nagymajtényi et al., 1994). It is still uncertain at which stages in the sensory pathway these changes happen and what is their nature. Considering the massive modulatory cholinergic innervation of the cortex (Johnston et al., 1981; Wainer et al., 1993) and the known influence of ACh on sensory evoked potentials (Rasmusson, 1993) the OP-induced changes could be ascribed to their anticholinesterase action (Koelle, 1981). Certain reports, however, indicate that "pharmacological" anticholinesterases, like physostigmine, do not fully mimic the acute neurotoxic action of OPs (Tomas and Gralewicz, 1992) and that anticholinergic drugs, like atropine, do not prevent or reverse that (Dési et al., 1991). Further, AChE can be present at the synapses in high excess (Koelle, 1992). Thus, only a massive blockage can be expected to show an effect, while in our experiment the brain AChE activity went down only to 85% of the control. And, where a clear cholinergic influence on sensory evoked activity was seen (Donoghue and Caroll, 1987; Rasmusson and Dykes, 1988) it was always facilitatory. Taken together, these data suggest that the decrease of the somatosensory evoked potential seen by us can be due to a non-cholinergic action of the OP.

The effect of the OP treatment on the hippocampal population spike, on the contrary, corresponds well with an increased cholinergic activity. Septo-hippocampal cholinergic fibres, ending mainly on the pyramidal neurons (Wainer et al., 1985) facilitate transmission (Krnjevic and Ropert, 1982). When, by means of the OP-induced AChE blockage, cholinergic influence is increased, a given stimulus can elicit a stronger response - as it was seen by us. A similar efffect of locally applied physostigmine had been published (Krnjevic and Ropert, 1982).

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