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CENTRAL NEUROTOXIC EFFECTS ELICITED WITH THREE ORGANOPHOSPHORUS COMPOUNDS: COMPARISON OF ACUTE AND SUBCHRONIC ADMINISTRATION

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Running head: comparison of acute and subchronic effects of organophosphates

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ABSTRACT

The neurotoxic effects of organophosphorous compounds had been amply investigated. There are, however, only a limited number of relevant data concerning neurotoxic effects of these substances exerted on central electrophysiological processes. The aim of the present study was to find out whether the effects of acute and subchronic administration of three organophosphorous compounds (dimethoate, parathion-methyl and dichlorvos) on the spontaneous and evoked cortical activity are comparable.

In the acute experiments, male Wistar rats were treated with 1/1 or 1/5 LD_{50} per os (parathion-methyl: 22.5 and 4.5 mg/kg; dimethoate: 700 and 140 mg/kg; dichlorvos: 98 and 19.6 mg/kg) and the changes of the cortical activity were recorded for at least 2.5 or 4.0 hours. In the subchronic administration, the animals were given 1/25 or 1/100 LD_{50} (parathion-methyl 0.9 and 0.225 mg/kg; dimethoate 28.0 and 7.0 mg/kg; dichlorvos 3.92 and 0.98 mg/kg) for 4, 8 or 12 weeks after which the animals were prepared and recording was done.

In acute experiments, dimethoate was the substance causing the strongest decrease of the ECoG whereas parathion-methyl induced the least changes. The duration of the evoked potentials was the most affected by dimethoate.

After 12 weeks of administration, parathion-methyl caused the largest alteration in the spontaneous and stimulus-evoked activity of the somatosensory and auditory focus while in the visual focus dichlorvos was the most effective.

The results of the study showed that the changes caused by the subchronic administration of the substances were sometimes equal to or larger than those caused by the acute large doses.

Key words: dimethoate, dichlorvos, ECoG, evoked potentials, organophosphates, parathion-methyl, rat

INTRODUCTION

Organophosphates (OP) are used, mostly as active ingredients of different insecticides, all over the world. The basis of their mechanism of action is the irreversible blockade of the acetylcholinesterase enzyme (15). Since this enzyme can be found also in mammals, these compounds can cause severe human intoxications (1,14).

The organophosphates affect the function of the central nervous system. A single large dose of sarin caused marked differences in the EEG frequency spectrum, even one year after the administration (7). Diisopropyl fluorophosphate, administered systemically, increased the spontaneous activity and reduced the light evoked activity in the superior

colliculi of rats (3). Intravenous administration of soman caused a 30 % depression of the performance of the visual cortex (4). Dimethoate, parathion-methyl and dichlorvos altered the spontaneous cortical activity and modified the latency and the duration of sensory evoked potentials either in acute (5,11,12) or in subchronic (6,10,13) administration.

The aim of this study was to find out whether the extent of the changes caused by three OPs, parathion-methyl (PM; ref.18), dichlorvos (DDVP; ref.16) and dimethoate (DIM; ref.17), given in low doses for a long period reaches the level of those caused by the same substances administered in a single large dose.

MATERIALS AND METHODS

Male Wistar rats were used, kept under standard conditions (temperature: 20-22 °C, relative humidity: 65-70 %, 12 hour light, 12 hour darkness period) and fed by standard chow and water ad libitum. One treatment group consisted of 10 rats.

Parathion-methyl (O,O-dimethyl O-4-nitrophenyl phosphorothioate), dimethoate (O,O-dimethyl-S-methylcarbamoylmethyl phosphorodithioate) and dichlorvos (2,2-dichlorvinyl dimethyl phosphate) wererchased at Huth AG, Hamburg, Germany, purity: >90 %.

The doses of the acute single administration (per os via gavage) were 1/1 and 1/5 LD_{50}, that is, parathion-methyl: 22.5 and 4.5 mg/kg; dimethoate: 700.0 and 140.0 mg/kg; dichlorvos: 98.0 and 19.6 mg/kg. Control rats were given saline.

For subchronic treatment for 12 weeks, the doses (given also by gavage) were 1/25 and 1/100 LD_{50} 5 times per week: parathion-methyl 0.9 and 0.225 mg/kg; dimethoate 28.0 and 7.0 mg/kg; dichlorvos 3.92 and 0.98 mg/kg.

In the acute experiments, the rats were anesthetized with urethane, 1000 mg/kg ip. (2), and a gastric tube was lead into their stomach. The animals' head was fixed in a stereotaxic frame and the surface of the left hemisphere was exposed. Half an hour later, spontaneous cortical activity (ECoG) from the primary somatosensory, visual and auditory foci, and then the somatosensory, visual and auditory evoked potentials (elicited by adequate stimulation) were recorded. Then, one of the above doses was given by gavage. The electrocorticogram and the evoked potentials were recorded every 30 minutes after administration up to 2.5 hours in case of 1/1 LD_{50}, and up to 4 hours in case of 1/5 LD_{50}.

In case of subchronic treatment, the rats were prepared on the day following the last administration as mentioned above (no without gastric tube, however). Spontaneous and stimulus-evoked activity was recorded once.

Of the ECoG, 5 min records were taken and analyzed for the activity distribution over the usual frequency bands, and the ECoG index (the ratio of the intensity of the slow (delta +

theta) and that of the fast (beta1 + beta2) bands) calculated. On the evoked activity, latency and duration were measured after averaging 50-50 potentials. Of all data, relative changes (treated/control) were calculated.

RESULTS

Acute treatment

In the acute experiments, the treatment with the higher, but not with the lower, dose of the substances induced typical signs of OP intoxication (salivation, etc.).

In these experiments, DIM (both doses) was the substance causing the strongest decrease of the ECoG-index whereas the changes induced by PM were the least. Practically all changes were significant ($p<0.05$).

Except for the auditory evoked potential and lower dose DDVP, the largest lengthening in the latency of the evoked potentials was caused by DIM. The smallest change was caused by PM except for the somatosensory evoked potential where the effect of high dose PM and DDVP were equal. The duration of the evoked potentials was the most increased by both doses of DIM except for the auditory evoked potential where the higher dose PM had the strongest effect. In both doses, PM had the weakest effect on the somatosensory, and DDVP on the visual evoked potentials.

Subchronic treatment for 12 weeks

No toxic symptoms were ever seen during and after the subchronic treatments. The body and organ masses had no significant alterations either.

In case of the high dose, PM caused the largest ECoG-index decrease in the somatosensory and auditory foci ($p<0.01$) while in the visual focus DDVP was the most effective ($p<0.001$). DIM and DDVP had equally weak effect on the index of the auditory cortex. In the low dose, PM had the smallest effect on the index in the somatosensory and visual foci while DDVP was the least effective on the index of the auditory focus. The index of the somatosensory cortex was the most altered by DIM, that of the visual cortex by DDVP and that of the auditory cortex by PM.

PM caused the largest increase in the latency of the somatosensory and auditory evoked potentials following treatment with both doses ($p<0.01$), and high dose DDVP had the strongest effect on the latency of visual evoked potential ($p<0.01$). The latency of the somatosensory evoked potential was the least affected by DIM and that of the visual evoked potential by PM. On the auditory evoked potential, DIM and DDVP had an equally weak effect. In case of the lower dose, the effect of the three substances on the latency

of the visual evoked potential was equal. DIM had the weakest effect on the somatosensory, and DDVP on the auditory evoked potentials.
PM also had the largest effect on the duration of the somatosensory and auditory evoked potential in both doses ($p<0.001$), and the higher dose of DDVP, on the visual evoked potential ($p<0.01$). DIM was the least effective in both doses, except on the duration of the auditory evoked potential, where DDVP had the least effect.

Comparison of the relative changes

In most cases, PM in 12 weeks administration caused nearly the same changes as following its acute administration. The decrease in the ECoG index in all three cortical areas was, following 12 weeks treatment, larger than after acute administration.
The lengthening of the latency and duration of the visual evoked potential after 12 weeks treatment was nearly equal to that following acute administration. In the somatosensory and auditory foci, it was larger.
In case of subchronic treatment with DDVP, the decrease of the ECoG index in the somatosensory and visual foci was below that evoked by acute administration. In the evoked activity, the latency increase of the somatosensory and auditory evoked potentials, and the duration increase of the somatosensory evoked potential were similar to those induced by acute treatment. The duration increase of the visual evoked potential was larger than in case of acute administration.
With DIM, merely the latency increase of the auditory evoked potential was similar following acute or 12-week administration.

DISCUSSION

As it was found in earlier acute and subchronic experiments, the three organophosphorous compounds had marked influence on the spontaneous activity of the cerebral cortex and on the sensory evoked potentials (5,6,10,11,12). They caused a significant decrease of the ECoG index and an increase of the latency and duration of the evoked potentials. Cholinergic modulation plays an important role in the alterations of the spontaneous cortical activity (8,9). Human data (7) also evidence that a single large dose of an OP may cause significant changes of the EEG frequency spectrum. The acute administration can serve as a model for the human intoxication related to suicides and accidents whereas subchronic administration can model occupational human intoxication, e.g. that of pesticide workers.

In our investigation, the relative changes caused by acute or subchronic administration of DIM, PM and DDVP in cortical electrical phenomena were determined and the acute effects were compared to the subchronic ones. Following 12 weeks subchronic administration of the three test compounds, similar or larger alteration of the ECoG frequency spectrum was seen than that obtained by acute treatment. A similar relationship could be observed on the latency and duration of the evoked potentials. Comparing the relative alterations, PM seemed to be the most dangerous among the three substances, because its subchronic administration, in most cases, caused the same or larger level of alterations than the acute one. DDVP seemed to be less dangerous, since its subchronic administration caused less marked alterations which in less cases reached the level of the acute effects. Among the three substances, DIM seemed to be the least harmful compound, since the effect of its subchronic administration was mostly below that obtained by acute administration. These observations are in line with the in vivo toxicity data (LD_{50}).

Conclusion: Since, in animal experiment, the subchronic administration of OPs in moderate doses caused similar or larger alterations in the spontaneous and evoked activity of the brain than the acute treatment with higher doses, subchronic human intoxications may be as dangerous as acute ones. Therefore, safety rules should be strictly adhered to in order to avoid occupational intoxications. Non-invasive electrophysiological tests (such as EEG or evoked potential recording) should be included into monitoring of the exposed.

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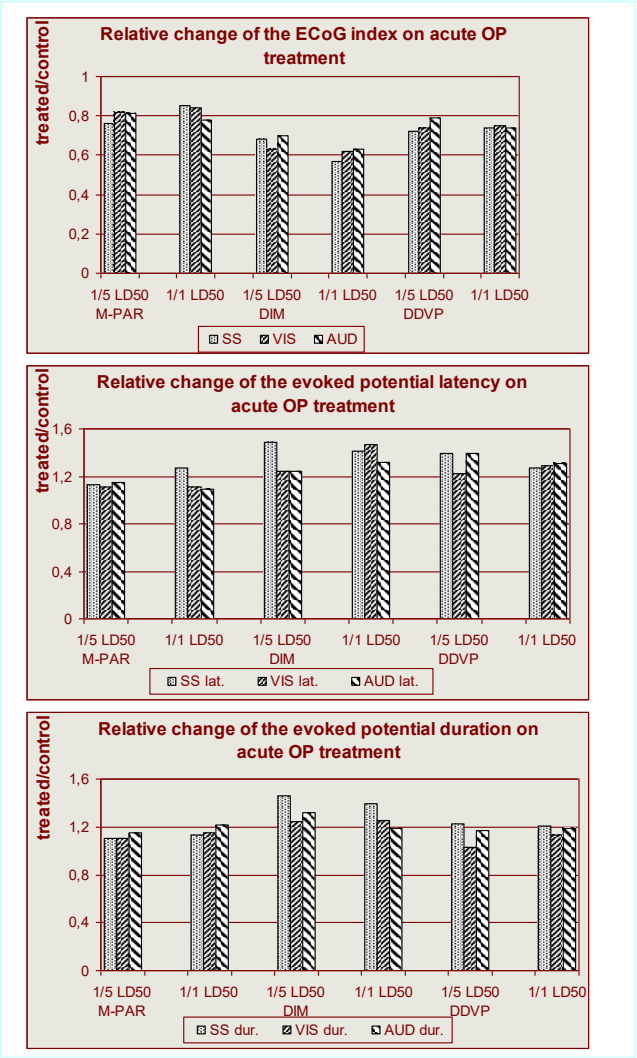

Figure 1. Relative changes of the ECoG index (top), evoked potential latency (middle) and duration (bottom) on acute administration of the three organophosphates. Abscissa: OPs and doses, ordinate: relative change, bar marking: cortical area.

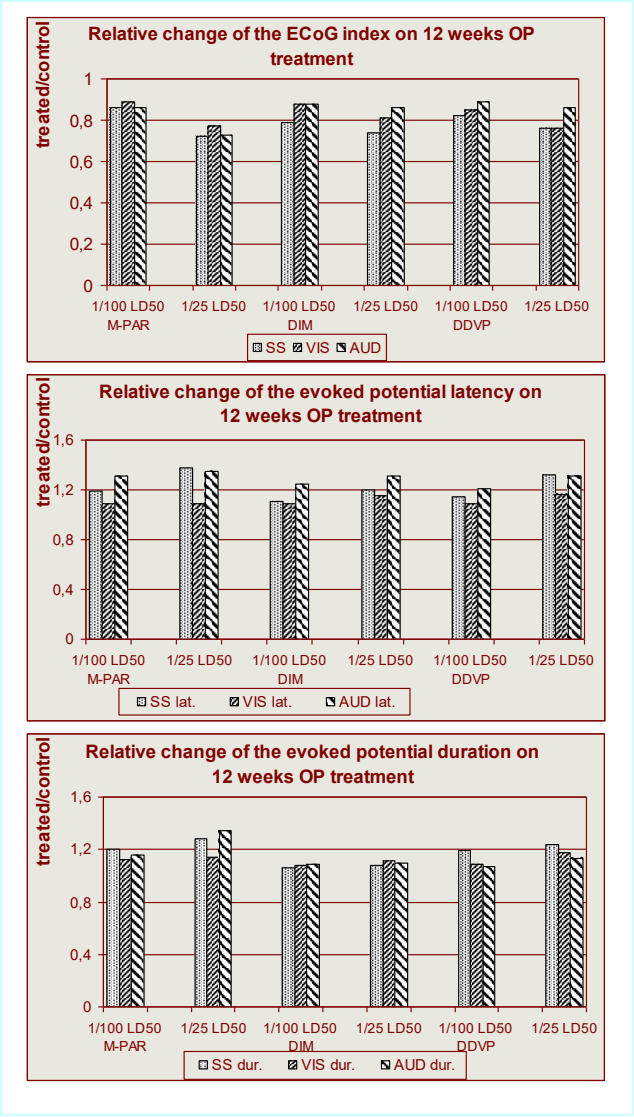

Figure 2. Relative changes of the ECoG index (top), evoked potential latency (middle) and duration (bottom) on 12 weeks subchronic administration of the three organophosphates. Displayed as in Fig. 1.