ELECTRORETINOGRAPHIC CHANGES INDUCED BY ORGANOPHOSPHORUS PESTICIDES IN RATS

Hideo YOSHIKAWA, Munehiro YOSHIDA and Ichiro HARA
Department of Public Health, Kansai Medical University, Fumizono-cho, Moriguchi, Osaka 570, Japan
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Abstract—Electroretinographic changes induced by organophosphorus pesticides (OPs) were studied in rats. Male Wistar rats were intraperitoneally injected with fenthion, chlorpyrifos, fenitrothion, dichlorvos or chlorfenvinphos at doses of 0.01 mmol/kg and/or 0.05 mmol/kg. The electroretinogram (ERG) was recorded at 5 hours and 2 days after the administration, and brain and retinchoroid cholinesterase (ChE) activities were assayed at 3 days after the injections. The brain and retinchoroid ChE activities were reduced in rats treated with the OPs. Notably, the reduction of ChE activities by fenthion, chlorpyrifos and dichlorvos were similar. The administration of OPs induced a change in the ERG, characterized by alteration of the amplitudes of a- and b-waves. Nevertheless the ChE activities in the brain and retinchoroid were inhibited by all of the OPs, the OPs affected the amplitude of ERG differently. Fenthion and chlorpyrifos decreased the amplitudes; dichlorvos and chlorfenvinphos increased; and fenitrothion transiently decreased at 5 hours but increased 2 days after the injection. These results indicate that a factor or factors other than inhibition of ChE activities contributes to the alteration of ERG induced by OPs.

Key words: Electroretinogram, organophosphorus pesticide, fenthion, chlorpyrifos, fenitrothion, dichlorvos, chlorfenvinphos, cholinesterase, ocular toxicity.

INTRODUCTION

Exposure of animals to organophosphorus pesticides (OPs) causes inhibition of cholinesterase (ChE) activities, and results in neurotoxic symptoms associated with the accumulation of acetylcholine. Recovery from toxic symptoms is believed to be parallel with return of ChE activity to normal level, especially in the central nervous system.

Correspondence: Hideo YOSHIKAWA at the above address.
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system (Duffy and Burchfield, 1980).

It has been reported that OPs produce ocular disorders such as decrease in visual acuity, visual field narrowing and optic nerve atrophy in persons exposed to these chemicals (Ishikawa, 1971; Ono et al., 1973). There are, however, little informations concerning the functional toxicity of OPs in visual systems.

Recently, the electroretinogram (ERG) has been used to assess retinal damage caused by exposure to chemical compounds (Chan and Hayes, 1985). Animal experiments have shown that administration of OPs induces ERG changes which are characterized by alteration of the amplitudes of a- and b-waves (Imai, 1975; Takeda et al., 1976; Carriacaburu et al., 1981). In these experiments, it has been postulated that the altered ERGs resulted from the inhibitory effect of OPs on ChE. However, the amplitude alteration caused by various OPs were not always the same direction; malathion, mevinphos and parathion were reported to decrease the amplitudes of a- and b-waves in mice (Carriacaburu et al., 1980, 1981), while, in cats, chlorfenvinphos increased the amplitudes (Takeda et al., 1976).

To clarify whether OPs decreases or increases the amplitudes of a- and b-waves, we administered different kinds of OPs to rats and measured the ERG. We also measured the ChE activities and discussed the relation of ERG alteration with the reduced ChE.

MATERIALS AND METHODS

Animal treatment:

Male Wistar rats, weighing 180 to 200 g, were housed in stainless-steel wire cages at a temperature of 22–24°C and a humidity of 60% in a room with a controlled 12 hour light (8 AM to 8 PM)-dark cycle, and were allowed free access to a commercial stock diet (Type MF, Oriental Yeast Co., Tokyo) and tap water. After one week of feeding, the rats were intraperitoneally injected with fenthion (O, O-dimethyl O-[3-methyl-4-(methylthio)phenyl]phosphorothioate), chlorpyrifos (O, O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate), fenitrothion (O, O-dimethyl O-(3-methyl-4-nitrophenyl phosphorothioate), dichlorvos (O, O-dimethyl O-(2,2-dichlorovinyl) phosphate) or chlorfenvinphos (O, O-dimethyl O-[2-chloro-1-(2,4-dichlorophenyl)vinyl] phosphate) at doses of 0.01 and/or 0.05 mmol/kg body weight. Fenthion was purchased from Gasukuro Kogyo Inc. (Tokyo), chlorpyrifos, fenitrothion and dichlorvos were obtained from Wako Pure Chemical Industries Co. (Osaka), and chlorfenvinphos was kindly supplied by Shell Kagaku Co. (Tokyo). Chemical structures of the OPs used in the experiment are listed in Fig. 1 together with molecular weights (MW) and median lethal dose (LD50). The OPs were dissolved in olive oil and control rats were injected with olive oil alone. All the injections were performed between 8 to 10 AM. ERG was recorded 5 hours and 2 days after the injection, and the ChE activities in the brain and retinochoroid were measured 3 days after injection.
ERG change by organophosphorus pesticide

Fenthion

\[
\text{MW: 278} \\
\text{LD}_{50}: \\
190-315
\]

Chlorpyrifos

\[
\text{MW: 350} \\
\text{LD}_{50}: \\
135-163
\]

Fenitrothion

\[
\text{MW: 277} \\
\text{LD}_{50}: \\
250-500
\]

Dichlorvos

\[
\text{MW: 221} \\
\text{LD}_{50}: \\
56-108
\]

Chlorfenvinphos

\[
\text{MW: 360} \\
\text{LD}_{50}: \\
10-39
\]

Fig. 1. Chemical structure, molecular weight (MW) and median lethal dose (LD$_{50}$) of organophosphorus pesticides used in this study. LD$_{50}$ (rat, oral) is expressed as mg/kg body weight, according to World Health Organization values (1986).
Recording of ERG:

The rats were anesthetized with intramuscular injections of 0.05 ml of DROLEPTAN* (2.5 mg/ml droperidole, Sankyo Co., Tokyo) and of 0.2 ml KETALAR 50* (57.6 mg/ml ketamine hydrochloride, Sankyo). Their pupils were dilated with topical administration of MYDRIN-P* (0.5% tropicamide and 0.5% phenylephrine hydrochloride, Santen Pharmaceutical Co., Osaka), and the rats were put in a dark room for 30 min. After the dark adaptation, the corneas were anesthetized with topical administration of BENOXIL* (0.4% oxybuprocaine hydrochloride, Santen Pharmaceutical) and a platinum-wire (0.5 mm i.d.) electrode was brought in contact with the cornea; a needle electrode subcutaneously inserted to the pariental region of the scalp served as a reference electrode. The left eye was stimulated every 10 sec with a 1.5 J xenon flash delivered from a photic stimulator (SLS-3100, Nihon Kohden Co., Tokyo) placed 30 cm from the eye. The low and high band-pass was set at 1 and 1000 Hz, respectively. The averaged ERG pattern of 10 successive trials was recorded by an electroretinograph with a computer (MEB-7102, Nihon Kohden).

Fig. 2 illustrates a typical ERG pattern of a control rat and the quantified parameters in the present study.

![Diagram of electroretinogram](image)

**Fig. 2.** A typical example of electroretinogram in rats.  
1, Amplitude of a-wave; 2, amplitude of b-wave; 3, latency of a-wave; 4, latency of b-wave.
ERG change by organophosphorus pesticide

Assays of ChE:

The brain was homogenized with 9 volumes of saline. After the lens and vitreous body were carefully removed from the eyes, the remaining retinochoroid was homogenized with 0.5 ml of saline. ChE activities in these homogenates were assayed by the methods of Voss and Sachsse (1970) using acetylthiocholine iodide as the substrate. Protein was determined by the methods described by Lowry et al. (1951).

Statistics:

Experimental data were examined by analysis of variance (ANOVA). When the F-value was significant (P<0.05), comparisons were made by the least significant difference (LSD) test to determine which value was significantly different from the control value (Snedecor and Cochran, 1969).

RESULTS

Table 1 shows the ChE activities in the brain and retinochoroid of the rats treated with various OPs at 3 days after the injections. All of the OPs significantly inhibited ChE activities. However, the extent of inhibition varied with the kind of OP. At a concentration of 0.01 mmol/kg, chlorfenvinphos showed 22% and 35% inhibition of brain and retinochoroid ChE activity, respectively, and dichlorvos also significantly inhibited the retinochoroid ChE. At the same dose, fenthion and chlorpyrifos did not show significant inhibition. When the dose level was increased to 0.05 mmol/kg, an obvious inhibition was also observed with fenthion, chlorpyrifos and fenitrothion, but the extent of inhibition varied. A higher

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose (mmol/kg)</th>
<th>Brain (unit/g protein)</th>
<th>Retinochoroid (unit/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>40.2±5.1</td>
<td>74.8±4.8</td>
</tr>
<tr>
<td>Fenthion</td>
<td>0.01</td>
<td>43.1±1.9</td>
<td>63.4±2.7</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>20.4±0.8***</td>
<td>40.6±5.2***</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>0.01</td>
<td>35.0±5.8</td>
<td>66.1±3.8</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>21.5±2.5***</td>
<td>41.5±6.8***</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>0.05</td>
<td>28.7±0.5*</td>
<td>65.4±6.1</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>0.01</td>
<td>38.4±3.4</td>
<td>58.1±2.6*</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>23.0±2.0***</td>
<td>42.1±11.4***</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>0.01</td>
<td>31.3±1.5*</td>
<td>48.3±5.9**</td>
</tr>
</tbody>
</table>

One unit of cholinesterase activity is expressed as 1 μmol thiocholine released per minute.

Values are means ± SE (n=5). Significant difference was observed from control group by ANOVA followed by the LSD test at P<0.05 (*), P<0.01 (**) or P<0.001 (***)

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inhibitory effect was observed with fenthion, chlordipryfos or dichlorvos than with fenitrothion; more than 40% inhibition to both ChE activities were observed with fenthion, chlordipryfos and dichlorvos, while 29 and 13% inhibition by fenitrothion were found in the brain and retinocochoroid enzymes, respectively.

Table 2 and 3 show the amplitudes and latencies of a- and b-waves 5 hours and 2 days after injection. Administration of 0.05 mmol/kg of fenthion or chlordipryfos changed the ERG, and a remarkable decrease in the amplitudes and a prolongation of

Table 2. Effect of organophosphorus pesticides on a-waves in the electroretinogram in rats.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Dose (mmol/kg)</th>
<th>Amplitude (μV)</th>
<th>Latency (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5 hours</td>
<td>2 days</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>273±11</td>
<td>273±5</td>
</tr>
<tr>
<td>Fenthion</td>
<td>0.01</td>
<td>—</td>
<td>248±11</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>118±23***  178±13***</td>
<td>18.5±1.6**  18.1±0.1*</td>
</tr>
<tr>
<td>Chlorpyritos</td>
<td>0.01</td>
<td>—</td>
<td>273±27</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>143±11***  218±14*</td>
<td>18.5±0.6**  18.1±0.9*</td>
</tr>
<tr>
<td>Fenitrothion</td>
<td>0.05</td>
<td>260±15</td>
<td>335±12**</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>0.01</td>
<td>—</td>
<td>350±11**</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>305±19</td>
<td>425±17***</td>
</tr>
<tr>
<td>Chlorfenvinphos</td>
<td>0.01</td>
<td>—</td>
<td>313±17</td>
</tr>
</tbody>
</table>

Electroretinograms were taken at 5 hours and 2 days after the injection of each pesticide. Values are means ± SE (n=5). Significant difference was observed from the control group by ANOVA followed by the LSD test at P<0.05 (*), P<0.01 (**) or P<0.001 (**).
ERG change by organophosphorus pesticide

the latencies of both a- and b-waves were observed at 5 hour post-injection. These alterations were still observed at 2 days post-injection, but at this time, the extent of alterations was not similar; the chlorpyrifos-induced decrease in the amplitudes tended to recover more rapidly to control levels than that induced by fenthion. Chlortefrinphos and dichlorvos had opposite effects from fenthion and chlorpyrifos on the ERG. Chlortefrinphos at 0.01 mmol/kg tended to increase the amplitudes at 2 days post-injection. In addition, administration of 0.01 or 0.05 mmol/kg of dichlorvos significantly increased the amplitudes of the two waves; 50% increase of amplitudes were observed 2 days after injection with 0.05 mmol/kg of this pesticide. The effect of fenitrothion on the ERG was complicated; fenitrothion at 0.05 mmol/kg decrease slightly the amplitudes of the two waves at 5 hours post-injection but significantly increase at 2 days after the injection.

DISCUSSION

The present study showed that OPs administered to rats caused a change in ERG amplitudes of a- and b-waves. The changes were classified into three types; decreased (fenthion and chlorpyrifos); increased (chlortefrinphos and dichlorvos); decreased at 5 hours after the injection and increased at 2 days post-injection (fenitrothion).

The alterations of the ERG amplitudes measured at 2 days were less than those measured at 5 hours, when the latter initially decreased, and were larger, when the latter increased. These facts lead us to consider the possibility that all of OPs share the property of producing transient decrease and then an increase in the amplitude of a- and b-waves. However, it has been reported that the increased amplitudes of a- and b-waves were observed even 30 min after the administration of chlortefrinphos in cats (Takeda et al., 1976). In addition, we did not observe the increased amplitudes of a- or b-waves in rats for 3 weeks after the administration of chlorpyrifos (Yoshikawa et al., 1990). Accordingly, the difference in the direction of the alteration observed at 5 hours after the administration of OPs, a decrease or an increase, is not explained simply by the phase shift of the alterations, or rather might be determined by the OPs administered themselves.

Previous studies (Imai, 1975; Takeda et al., 1976; Carricaburu et al., 1980, 1981) have described discrepant findings concerning the direction of the OP-induced alteration in the ERG as mentioned in the introduction. However, much attention has not been paid to this difference in the direction of altered ERG waves. They have postulated simply the increase or decrease in the amplitudes to be caused by the inhibitory effect of OPs on ChE. The present study showed that the brain and retinochoroid ChE were inhibited by the OPs administered. Especially, fenthion, chlorpyrifos and dichlorvos produced the similar extent of inhibition. However, fenthion and chlorpyrifos decreased the amplitudes and dichlorvos increased. These results indicate that a factor(s) other than the inhibition of ChE contributes to the
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shift of amplitudes of ERGs in rats administered OPs.

Generally, retinal degeneration causes decreased amplitudes and prolonged latencies of a- and b-waves in ERG (Watanabe and Miyake, 1984). Decrease of the amplitudes and prolongation of the latencies observed in the rats with fenthion or chlorpyrifos may be derived from the retinal degeneration caused by these pesticides.

ERG is also influenced by several chemical substances other than OPs. Among these substances, trichloroethylene (TCE), with a chemical structure of \( \text{CHCl} = \text{CCl}_2 \), has been reported to induce a changed ERG with increased amplitudes of a- and b-waves similar to dichlorvos or chlorfenvinphos (Watanabe and Miyake 1984). A common structure is found between TCE and dichlorvos and chlorfenvinphos; chlorovinyl group exists in all these compounds. These facts lead us to speculate that a chemical structure other than the phosphate ester group in OPs may contribute to the OPs-induced ERG change.

Abnormal ERGs, probably caused by OPs, were found in workers engaged in agriculture or pest control operation (Ishikawa, 1973; Yoshikawa et al., unpublished observation). For persons handling OPs occupationally, ERG is thought to be an available measure for evaluation of chemical-induced retinal damage. However, most OP-exposed workers handle many kinds of OPs for a long time. Further study is necessary to elucidate chronic effects of mixed exposure to plural OPs on the visual system.

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