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Experimental Model Studies of Pesticide Exposure

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Abstract: I. Dési, L. Nagymajtényi, A. Papp and H. Schulz. Experimental Model Studies of Pesticide Exposure. *Neurotoxicology* **19**(4-5):611-616, 1998. *The neurotoxic effects of Dimethoate (Dim), Dichlorvos (DDVP) and Methyl-Parathion (MP) respectively were investigated on the central nervous system (CNS) and peripheral nervous system (PNS) of rats after different treatment schedules at the macro and single unit cell level. At the macro investigations 1/25, 1/50 and 1/100 of the respective LD50 values of each pesticide were administered to different groups by gavage daily in the following programs: Pregnancy variation (P) to females from 5th to 15th days of pregnancy; Pregnancy and lactation variation (P+L): to females as above and during lactation for 4 weeks; Pregnancy+lactation+post weaning variation (P+L+P) as above plus to the young male rats (F1 generation) up to 8 weeks. Neurotoxicological investigations were conducted on the F1 rats at the age of 12 weeks. Spontaneous electrocorticograms (ECoG) were recorded on the anesthetized rats from the somatosensory, visual and auditory cortex. Cortical evoked potentials (EP) were recorded from the same areas subsequently. Conduction velocity and refractory periods of the tail nerve was investigated. Treatment by Dim, DDVP and MP during P and P+L of the mothers did not influence the bioelectric activity of the offsprings significantly. The same treatment by the P+L+P programme, resulted in significant changes. Frequency of the spontaneous ECoG waves grew significantly in all dose groups of P+L+P group. Latency time become shorter after somatosensory. light or acoustic stimuli respectively on one hand and the beginning of the of answer of these by the evoked potential (EP) waves on the other hand. Conduction velocity of the tail nerve diminished, refractory periods grew dose dependently and significantly at the P+L+P programs with all the three pesticides. Cortical single unit activity was studied after the i.p administration of 1/5 LD50 of the three organophosphates (OP). The decrease of the firing frequencies was observed. The amplitude of the hippocampal population spikes increased. The changes observed in these studies point toward a higher excitation state of the CNS and a disturbed conduction of the nervous impulses of the peripheral nerves. These results could be taken into consideration when deciding on human contaminations by OP-s. © 1998 Intox Press, Inc.*

Key Words: Dimethoate, Dichlorvos, Methyl-Parathion, CNS, PNS, ECoG, Unit-Potentials, Hippocampal Population Spikes

INTRODUCTION

Pesticides cannot be abolished because this would cause famine in the world (Knutson *et al.*, 1990). The damaging effects of the pesticides, on the other hand, make it necessary to thoroughly check their respective toxicity (Conway and Pretty 1991, Dési 1994, Dési *et al.*, 1997, Hammond 1994). It is necessary to detect in animal experiments, the mild, functional changes caused by pesticides at an early stage of the intoxication and the discovery of the fine modes of the pesticide action (Koelle 1992, IPCS 1986).

There are some rather sensitive periods in life as the gravidity, the lactation and the early epoch of life when mammals are greatly sensitive toward intoxication. Our aim was to study the consequences of moderate pesticide exposure exerted to experimental rats during these sensitive periods.

The bioelectric functions of the central and peripheral nervous system was studied, since it is one of

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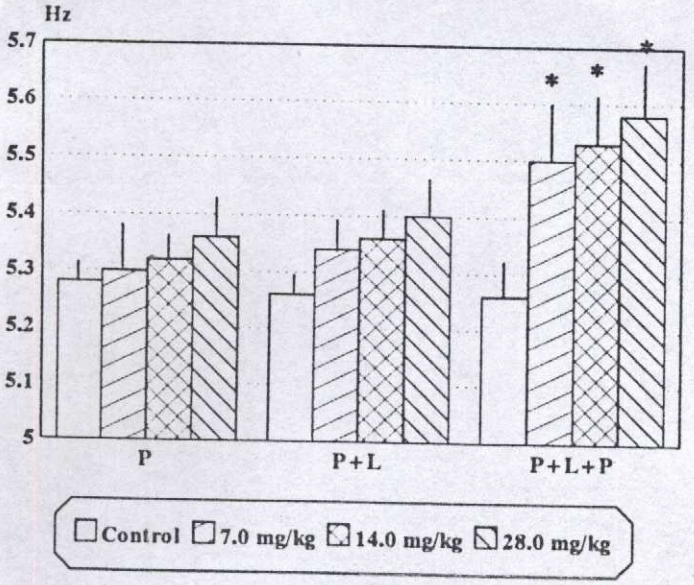

FIG. 1. Changes of the ECoG mean frequency of the somatosensory area after Dimethoate (DIM) treatment. Ordinate: mean frequency. Abscissa: P: pregnancy; P+L: pregnancy+lactation; P+L+P: pregnancy+lactation+post weaning variation. Error bar: S.D. Insert: doses, significance: * p<0.05, ** p<0.01, *** p<0.001.

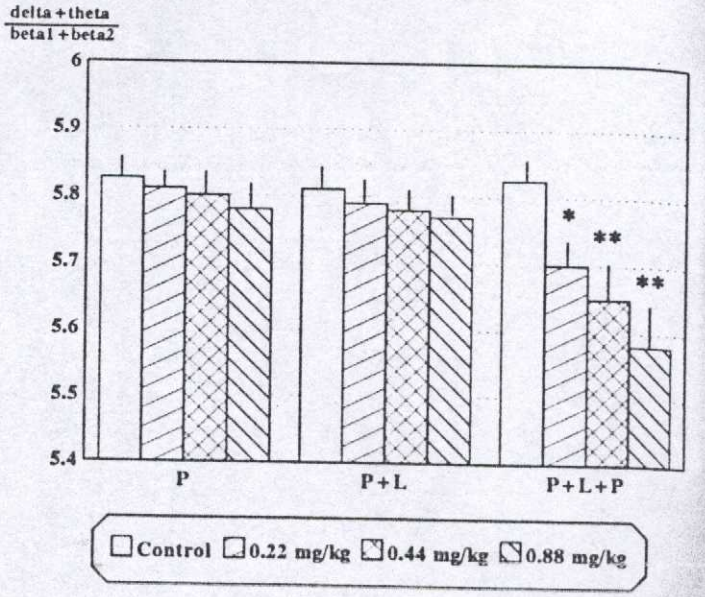

FIG. 2. Changes of the ECoG index of the somatosensory area after Methyl-Parathion (MP) treatment. Ordinate: index values. Other markings as in Fig. 1.

the sensitive biomarkers. Beside determining the macro functions (electrocorticogram and evoked potentials) additional experiments were conducted on one neuron level to understand the disruption of the subtle processes of the nervous system to these pesticides.

MATERIALS AND METHODS

Specific pathogen free (SPF) outbred Wistar rats were used in these studies. (Laboratory Animal Breeding Farm, Gödöllö, Hungary). The rats were kept at standard condition: temperature of the animal house 20-22 °C; humidity 60-70%. A 12-12 light-dark cycle was maintained. The animals were fed with standard rat food and water ad libitum. All procedures with the rats were carried out as per the regulations approved by the Ethics Commission of the University.

Pesticides

Dimethoate (Dim, O,O-dimethyl-S-(N-methylcarbamyl)-methyl-ditiophosphate) LD50: 700 mg/kg; Dichlorvos (DDVP, 2,2-dichlorvinyl-O,O-dimethylphosphate) LD50 98 mg/kg; methyl-Parathion (MP), O,O-dimethyl-O-p-nitrophenil-thiohosphate) LD50: 22 mg/kg. All supplied by Huth AG Hamburg FRG with a purity of 97-98 %.

Administration of the pesticides: 1/100, 1/50 and 1/25 of their respective LD50 value P.O. by gavage daily, (Dim: 7.0, 14.0, 28.0 mg/kg, DDVP: 0.98, 1.96, 3.92 mg/kg, PM: 0.22, 0.44, 0.88 mg/kg) The pesticides were dissolved in saline, added to it Tween 80. Controls received saline only, with Tween 80. Administration of the pesticides was done according to the following protocols:

(a) *Pregnancy (P) variation.* At the age of 11-12 weeks, the animals were mated, two females and one male per cage, to obtain the F_1 generation. The pregnant females were treated from the 5th to the 15th day of pregnancy (day 0: vaginal plug).

(b) *Pregnancy+lactation (P+L) variation*: Parent females were treated as in "a", plus during lactation, from the 2nd day after delivery until separation of the offsprings at their age of 4 weeks.

(c) *Pregnancy+lactation+post weaning (P+L+P) variation:* The parent females were treated as in "b" plus the weaned F_1 young males treated further for 8 weeks.

The neurotoxicological investigations were made on the F_1 generation, using 10 males per group at their ages of 11-12 weeks. The experimental and control rats were anesthetized with 1000 mg/kg urethane (Bowman and Rans 1980), and placed in stereotaxic instrument. Silver electrodes were placed on the exposed primary somatosensory, visual and auditory centers. The electrocorticogram (ECoG) was recorded a half an hour later. Parameters calculated by our electronic set up were mean frequency, the ECoG index (the ratio of the slow and fast bands of the spectral power: delta+theta/beta1+beta2) (Dési, 1983).

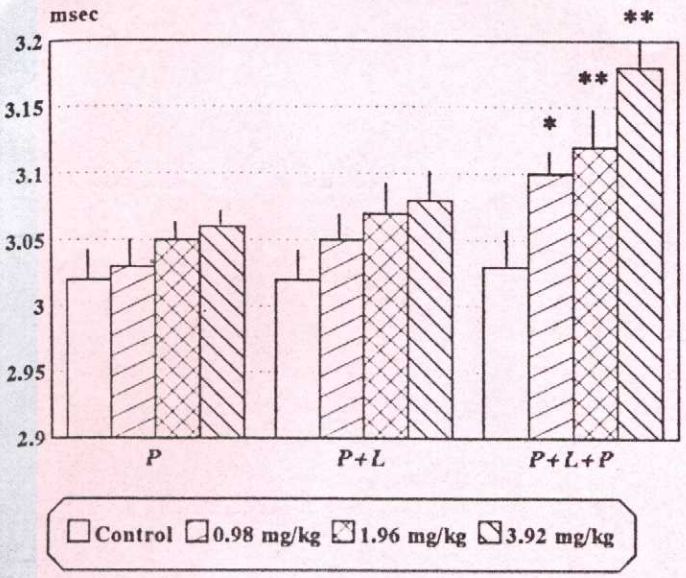

FIG 3. Changes of the latency of one selected wave of the evoked potentials, somatosensory N1 wave. Ordinate: latency time. Other markings as in Fig. 1.

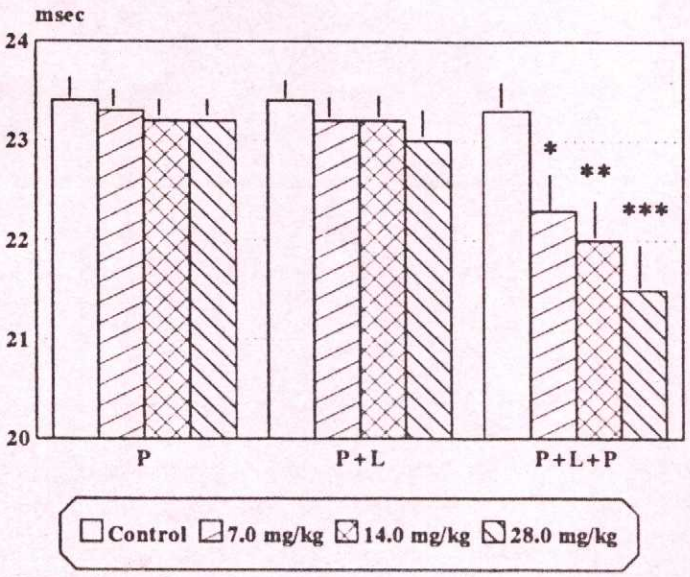

FIG. 4. Changes of the conduction velocity in the rat tail nerve after Dimethoate treatment. Ordinate: velocity in msec. Other markings as in Fig. 1.

Evoked potentials (EP) were recorded through the same electrodes.

Somatosensory stimulations were done via electrodes inserted into the nasal part of the skin.

Parameters

1 Hz, 3 V, 0,2 msec. Visual stimulation: flashes 1 Hz, 60 lux via an optical fibre put to the eye. Acoustic stimulation: clics 1 Hz, 60 dB through an earphone placed in the ear. After application of 50 stimuli per rat of each modality the averaging of the values was done by a Cambridge Electronics computer programme. The latencies of the EP-s were measured (somatosensory stimulation: N1 wave, others: N2 wave).

Conduction velocity of the tail nerve was studied by the modified Miyoshy and Goto (1973) method, whereas the time span of the relative and absolute refractory period was determined according to the method of Anda *et al.*, (1984).

Single unit activity was recorded extracellularly by a glass microelectrode which was advanced into the somatosensory area. Signals of extracellular activity were lead into an AC amplifier and visualized on a storage scope screen and fed into an interface for storage and analysis. After recording of control activity 1/5 LD_{50} of the three pesticides respectively was injected i.p. Following pesticide administration recordings were made at 5 min intervals. Thus each animal served as its own control. Evaluation of the single unit activity was based on the measurement of the interspike intervals (ISI), the time gaps between two successive single unit discharges. The program yielded the average value and a distribution histogram.

The evoking and recording of hippocampal population spikes after administration of 1/5 LD i.p. of the organophosphates was performed as part of measurement of unit activity (Papp *et al.*, 1996). A bipolar stimulating electrode was positioned to the perforant path,

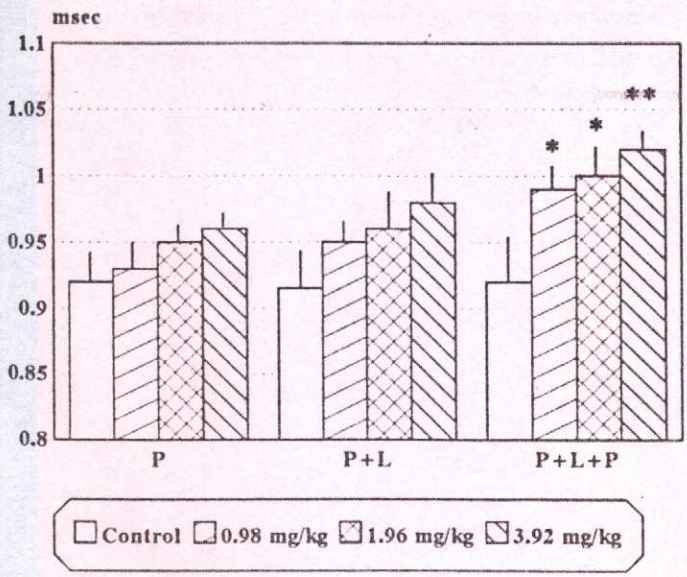

FIG. 5. Changes of the absolute refractory period of the rat tail nerve after Dichlorvos (DDVP) administration. Ordinate the period in msec. Other markings as in Fig. 1

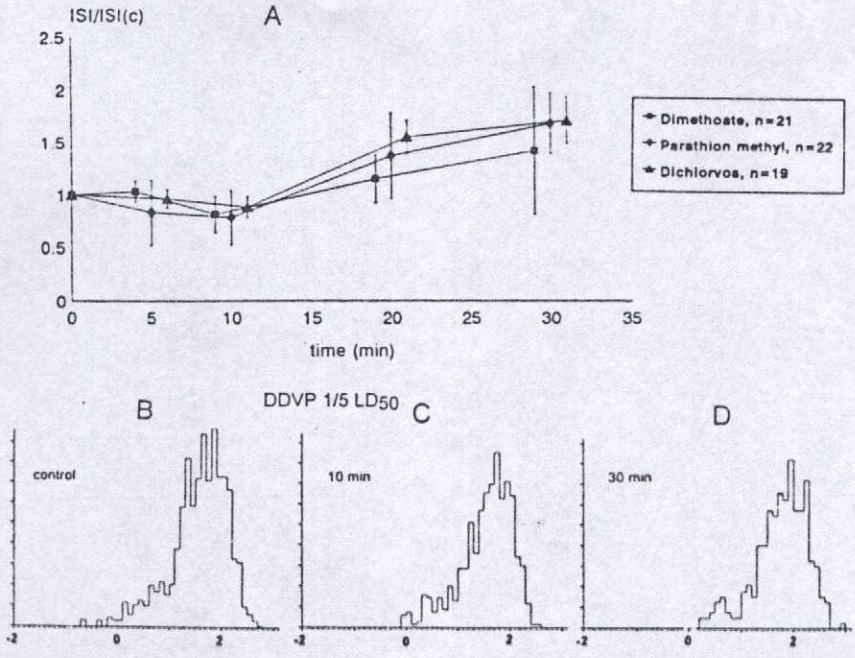

FIG 6. Changes of the interspike intervals (ISI) of single unit firing after ip administration of 1/5 LD of the organophosphates. A: Averaged relative changes, with S.D. of the mean ISI. significancy $p<0.05$. Ordinate: ISI experimental/ISI control values. Abscissa: time after treatment. End of the control period at 0 min. n: No of animals used. B.C. D: ISI histograms demonstrating the difference in control state (B) vs. 10 (C) and 25 (D) min under DDVP influence. Abscissa: ISI in log msec, one bin/tick; ordinate: counts per bin.

the recording glass microelectrode was placed in the CA1 region. The perforant path was stimulated by square pulses 1 mA, 0.5 ms, 0.1 Hz. The low frequency was chosen to avoid facilitation. A train of 20 stimuli was given every 10 minutes. The evoked population spikes were recorded and averaged after amplification. From the data measured later on the monitor screen basic statistical parameters were calculated for the whole groups, 15 animals each.

Statistics

For statistical evaluation of treatment effects we used multivariate ANOVA following *square root* transformation of the data and equal cell content with an 3 x 4 design (treatment x dose). *Post hoc* analysis was done by subsequent Scheffé's test to determine between group differences. In cases of non-normality the Kruskal-Walis ANOVA was used. The probability level was at $p<0.05$.

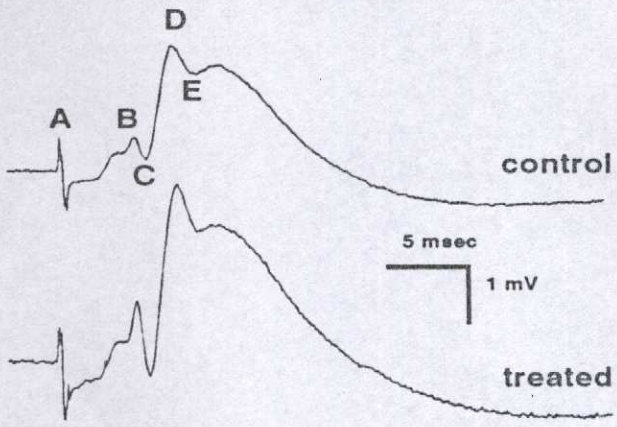

FIG 7. Hippocampal population spike recorded from the CA1 area of the rat. Upper trace: control, lower trace: 30 min after i.p. administration of 1/5 LD50 DDVP.

RESULTS

The F_1 rats were continuously observed for symptoms of intoxication: salivation, muscle tone, etc., and gain in body weight. No differences between the treated and the controls were found in the above mentioned parameters.

At CNS level there were important differences. The ECoG frequencies increased after the administrations of the different pesticides. These increases were visible but not significant with the P and P+L groups and were significant ($p<0.05$) at all the three doses in the P+L+P group (Fig 1).

The changes of the ECoG indices demonstrated the diminishing of the slow and increasing of the fast wave bands. At treatments with all the three OP-s these shifts

were similar as shown in Fig. 1, but the significances were stronger (LD_{50} 1/25 and LD_{50} $p<0.01$; 1/100 $p<0.05$) (Fig. 2).

The latency times of the EP-s became longer significantly at all the three doses of P+L+P programs after Dim, DDVP and PM treatment (LD_{50} 1/25 and 1/50 $p<0.05$, LD_{50} 1/100 $p<0.01$) (Fig 3).

The conduction velocity of the tail nerve slowed down significantly in rats treated with 3 doses with all three pesticides in the P+L+P group (LD_{50} 1/25 $p<0.001$, LD_{50} 1/50 $p<0.01$ and 1/100 $p<0.05$) (Fig 4). Relative and absolute refractory times of the peripheral nerve became longer in similar way (LD_{50} 1/25 $p<0.01$; 1/50 and LD_{50} 1/100 $p<0.05$) (Fig 5).

The effects of all the three pesticides on the unit activity manifested itself first during a brief period of slight shortening of the mean ISI, a transient increase of the firing frequency. Afterward it was seen a lengthening of the mean ISI, that is, the decrease of the firing frequency. This effect was significant ($p<0.05$) (Fig 6A).

The ISI histograms showed a marked difference between control state and under pesticide influence (Fig 6B,C,D)

When recording the hippocampal population spikes, after ten minutes of administering the pesticides the peak to peak amplitude of the spike increased (Fig. 7) for about 30 minutes and remained at that level up to the end of the recording. Latency and duration of the population spikes did not change.

DISCUSSION

The gravidity, lactation and the early days of life are rather sensitive periods to the effects of chemicals. The embryo and the newborn are more susceptible to foreign substances than the adults. Chemical contaminations, even mild ones, occurred during pre and postnatal periods may cause lasting damages of the developing nervous system, which probably would manifest itself completely at later periods of life (Nyakas 1992).

In the present study, we examined the pre and postnatal effects of those organophosphorus pesticides, the influence of which on adult rat population was already studied by us (Dési *et al.*, 1991; Dési *et al.*, 1944; Nagymajtényi *et al.*, 1994a; Nagymajtényi *et al.*, 1994b).

We found in our recent neurotoxicological experiments that the three investigated organophosphate exerted very similar effects on the CNS, the PNS and on the one unit (cell) activity. In the CNS investigations of ECoG as well as on the EP-s the results obtained from somatosensory, visual or auditory areas of the brain, were identical too. These findings suggest a general effect induced by the pesticides, probably an influence on the acetylcholine-cholinesterase system (Koelle 1992).

Treatment during pregnancy (P) and pregnancy+lactation (P+L) of the mothers did not influence the bioelectric activity of the offsprings. One could postulate different possibilities for the cause of this phenomenon: (a) The larger doses of these substances may not cross through the blood/placenta barrier, and the milk respectively at least in the present experimental conditions; (b) the offsprings might not yet have developed sensitive sites towards the intoxicating agents and (c) during the interval between treatment and ECoG the offsprings might have recovered from the mild intoxication. Naturally, these assumptions can only be verified by further investigations.

The mothers, exposed during pregnancy (P) and pregnancy and lactation (P+L) transferred fine, non-significant disturbances in the CNS to the F_1 generation. This may give rise to the speculations that either the amount of pesticides entered via the maternal blood and/or milk into the offsprings is small, or sensitive sites might have been already developed, or some other effect began to manifest itself in spite of the interval between treatment and investigation.

The treatment of F_1 generations, resulted in dose dependent changes in both the CNS and the PNS of these age groups. The changes pointed toward a higher excitation state of the CNS and a disturbed conduction of the nervous impulses of the peripheral nerves. Similar alterations were found in our experiments conducted with more aged rats (Dési, 1983; Dési *et al.*, 1991; Nagymajtényi *et al.*, 1994a,b; Nagymajtényi *et al.*, 1995).

In our earlier investigations (Nagymajtényi *et al.*, 1995), administering the same doses of DDVP as in the present experiment to adult rats, the activity of brain cholinesterase was significantly inhibited only at the highest dose.

There were disturbances in the CNS at one neuron level of the rats exposed to OP-s (Gralewitz *et al.*, 1991). The firing of the cortical neurons in our experiments deteriorated as well. The hippocampal population spikes grew in amplitude. As the amplitude depends on the number of units, firing together simultaneously contributed to increased firing activity. To see if this effect was related to the anticholinesterase activity of the compounds, we compared this outcome to the efficiency of physostigmin a standard anticholinesterase agent.

The effect of latter was nearly identical to that of the organophosphates (Data not shown).

ACKNOWLEDGMENT

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