FUNCTIONAL NEUROTOXIC EFFECTS OF COMBINED ORAL ADMINISTRATION OF INSECTICIDES TO RATS DURING INTRA- AND EXTRAUTERINE DEVELOPMENT

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Functional neurotoxic effects of combined oral administration of insecticides to rats during intra- and extrauterine development - Zsuzsanna Lengyel, Zita Fazakas, András Papp - Homeostasis 43, 4, 2005 - Most of the modern insecticides act on the nervous system. Although there have been extensive toxicological studies with the agents themselves, knowledge of the consequences of combined effects is as yet insufficient. The aim of the present study was to record and analyze the alterations in the cortical electrical activity of rats treated with combinations of widely used insecticides during development. Three insecticides were used in 1/25LD_{50} dose: dimethoate (DIM, an organophosphate), propoxur (PRP, a carbamate) and cypermethrin (CYP, a pyrethroid), given in double (DIM-PRP, DIM-CYP, PRP-CYP) and triple (DIM-PRP-CYP) combinations in the following manner: Daily by gavage, from day 5 to 15 during pregnancy (P protocol), or from day 5 to 15 during pregnancy + for 4 weeks of lactation (P+L protocol), or from day 5 to 15 during pregnancy + for 4 weeks of lactation + the male offspring (F1 generation) treated for further 8 weeks post-weaning (P+L+P protocol). The electrophysiological parameters were investigated in the F1 male offspring (10 animals per group) at their age of 12 weeks. General toxic effects were monitored by the animals' weight gain. - For electrophysiological recording, the rats' left hemisphere was exposed in urethane anaesthesia, and spontaneous and stimulus-evoked activity was recorded from the primary somatosensory, visual and auditory area. The effect on the spontaneous activity was moderate. In all treatments involving DIM, fast waves were relatively increased, with the strongest effect seen in the DIM-CYP group. In the DIM-PRP and DIM-CYP combinations, the effect with the P treatment protocol was opposite to that obtained with P+L or P+L+P. Latency of the somatosensory evoke potential was significantly shortened by the PRP-CYP and DIM-PRP-CYP combination when given during both pre- and postnatal development. Latency of the visual evoked potential was significantly lengthened by all combinations, mostly irrespective of the timing of treatment. - These results emphasize the negative effect of environmental toxicants on the developing nervous system and show the need for further studies in combination toxicology.

INTRODUCTION

Pest control is a major part of modern agriculture. Most of the insecticides in use act on the nervous system. Occupational, or food- and water-borne, exposure, and the incomplete target specificity of the agents, may result in human nervous system damage. Due to the numerous pesticide agents applied, combined exposure, leading to various, as yet unknown, interactions, is likely.

Organophosphates (WHO 1986b) are known to cause permanent inhibition of acetylcholinesterase (Koelle 1992). In human OP intoxication, a variety of nervous system effects have been observed, first of all EEG abnormalities (Muttray et al. 1996). Dimethoate (DIM) has been chosen as an OP to our study because of its moderate human toxicity (WHO 1989a) and widespread use in a lot of countries.

Carbamates (WHO 1986a) are another group of insecticide agents with cholinesterase blocking as main action (Alvares 1992). This effect of carbamates is, however, reversible. Propoxur (PRP), the carbamate used in the present study, is applied mainly in household pest control and for residual spraying in malaria eradication programs. In humans, the symptoms of PRP poisoning are typical for cholinergic overweight (WHO/FAO 1989), although atropine-like effects following PRP exposure are also known (Kobayashi et al 1994). The similarity of the functional neurotoxicity of PRP and an OP (methyl parathion) was demonstrated by Institóris et al (2004).

Pyrethroids are widely used as insecticides because of their high insecticidal potency, low mammalian toxicity and biodegradability (WHO 1989b). The agent studied, cypermethrin (CYP), belongs to the type II pyrethroids which have mostly central action (Leahey 1985).

Although the mentioned insecticide agents are in widespread use so that multiple occupational exposure is possible, and simultaneous presence of residues in the environment cannot be excluded, information on their simultaneous effects and interactions is minimal.

METHODS

The experiment was started with pregnant female Wistar rats (250 g body weight), treated via gavage as shown in Table 1. The treatment protocols applied were:

P protocol - dams (5 per group) treated in the 5th to 15th day of pregnancy;
P+L protocol - like P, plus dams treated during the 4 weeks of lactation;
P+L+P protocol - like P+L, plus the male offspring treated for 8 weeks after weaning.

At their 12 weeks of age, the young males (10 per group and protocol, altogether 150) were anesthetized by urethane (1000 mg/kg p.) and prepared for electrophysiological recording by exposing the left hemisphere. Lidocaine (10 %) was applied on the wounds and liquid paraffin on the exposed dura. Following ca. 30 min recovery, silver recording electrodes was placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas, and electrocorticogram (ECoG) was recorded simultaneously from these sites for 6 minutes. Then, sensory stimuli (in a series of 50 with 1 Hz repetition frequency) were applied and the cortical evoked potentials (EPs) recorded from the same sites. The somatosensory stimulus was a weak electric shock to the whiskers, visual stimulus, a flash, and auditory stimulus, a click. The ECoG analysis provided the frequency power spectrum by bands (delta to gamma). On the cortical EPs, latency and duration of the main waves was measured manually after averaging. From the tail nerve, compound action potentials, elicited by electric stimulation, were recorded, and conduction velocity as well as refractory period was determined. See Dési et al, 1999. for details of the electrophysiological technique used.

Analysis of the ECoG records provided the frequency power spectrum by bands (delta to gamma; Kandel and Schwartz, 1985). On the cortical evoked responses, latency and duration of the main waves was measured manually after averaging. From the tail nerve records, conduction velocity and relative refractory period was determined as described in Dési and Nagymajtényi, 1999. All recording and analysis was PC-based, using the NEUROSYS 1.11 software (Experimetria, U.K.).

Statistical analysis was done by one-way ANOVA and LSD post-hoc test ($p<0.05$) after the Kolmogorov-Smirnov normality check (SPSS 9.0).

Group	Treatment	Dose (mg/kg body weight)
CON	vehicle control	--
CP	cypermethrin + propoxur	3.4 + 22.2
DC	dimethoate + cypermethrin	28.2 + 22.2
DP	dimethoate + propoxur	28.2 + 3.4
DPC	dimethoate + propoxur + cypermethrin	28.2 + 3.4 + 22.2

Table 1. Group codes, treatment and doses (all doses are 1/25 LD_{50}). The substances were dissolved in sunflower oil and given by gavage.

RESULTS

Tested insecticides in doses of 1/25 LD50 had some clear-cut effects on the **spontaneous cortical activity** (electrocorticogram, see table 2).

P protocol: in the rats treated by DC the activity in the delta and theta band increased in all three cortical areas. In the DP group, activity in beta2 and gamma bands decreased in the visual area. In the DPC group, delta and theta activities increased in the somatosensory area, and gamma decreased in all three areas.

PL protocol: in DC and DPC treated rats, delta and theta activities decreased, and gamma activity increased. In the DPC group, also beta2 activity increased significantly in somatosensory and auditory areas.

PLP protocol: in the DC and DP groups, delta activity decreased, but increased in DPC group in all cortical areas. Gamma activity increased (non-significantly) in the CP group, but decreased in all other groups. In the visual area, alpha activity decreased in nearly all treatment groups.

Latency was the parameter of the cortical evoked responses most affected by the insecticides (Fig 1). In the P protocol, DP and DPC treatment caused the strongest latency increase. In case of PL and PLP protocol, all combinations had an effect, most pronounced in the visual cortex. The duration of the responses showed the biggest changes in the auditory cortex (Fig. 2).

In the tail nerve, conduction velocity was significantly altered in the CP group (PL and PLP protocol) and in the DPC group (P and PL protocol). The refractory period showed no effect (Fig. 3).

CONCLUSION

The results showed that in the treatment protocols applied, including both the pre- and postnatal development, the neurotoxic effect of the pesticides could be detected and analyzed. The effect of exposure to combinations of insecticide agents was seen primarily on the cortical activity, and the effects were partly differed in each area. The interactions seen, first of all the non-additive ones, indicated that the toxicology of pesticide combinations deserves increased attention.

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Table 2. Relative power spectrum of the electrocorticogram according to the standard EEG bands.

	Somatosensory					Visual					Auditory				
	CON	CP	DC	DP	DPC	CON	CP	DC	DP	DPC	CON	CP	DC	DP	DPC
protocol P															
Delta	24	25	27*	27	26*	27	27	36*	32	26	28	27	33*	30	34*
Theta	21	23	27*	22	30*	19	23	24*	20	31*	18	21*	22*	18	22
Alpha	16	17	16	14	18	13	14	13	12	17	14	16	15	14	16
Beta1	11	12	10	10*	10	10	10	8	8	9	10	12	11	10	11
Beta2	13	15	13	13	11	14	14	12	11*	11	13	15	13	12	12
Gamma	14	14	11	11	9*	16	15	11	11*	10*	14	12	11*	11	9*
PL protocol															
Delta	24	27*	20*	25	14*	33	34	23*	29	21*	31	31	21*	26	16*
Theta	25	24	18*	22	17*	23	23	17*	22	16*	21	22	17*	20	16*
Alpha	18	17	15	16	16	14	14	13	14	14	15	16	15	16	15
Beta1	11	11	11	11	13	9	9	10	10	11	11	10	11	11	13
Beta2	15	14	15	15	24*	14	12	16	15	20	14	14	15	16	24*
Gamma	12	11	16	15	25*	13	12	17	16*	24*	13	12	15	15	25*
PLP protocol															
Delta	27	26	22*	23*	38*	31	31	32	30	35*	31	28	28	27	39*
Theta	24	21	21	23	25	21	18	20	23	21	20	18*	19	20	25*
Alpha	16	15	17	17	13	14	12*	12*	14	11*	16	14	15	15	11*
Beta1	10	10	11	10	8*	10	9	9	9	10	11	10	11	11	7*
Beta2	14	13	15	13	12	15	14	13	12	15	15	15	15	14	11
Gamma	13	14	13	12	10*	14	16	13	12	13	12	15*	13	13	9*

The numbers in the cells are group means (n=10) of the percentage of the given band in the total ECoG, rounded off to integer. *: $p<0.05$ vs. the same band in the control, in the same area.

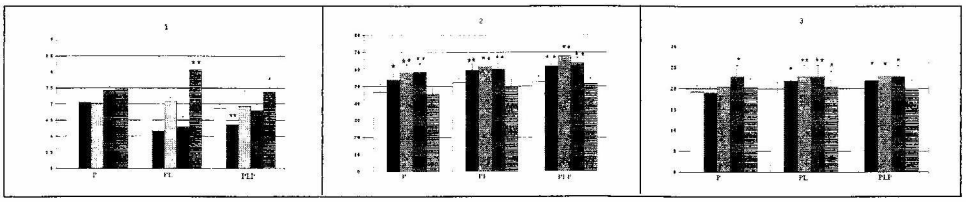

Figure 1: Latency of the cortical evoked potentials (1, somatosensory; 2, visual; 3, auditory) in control rats and in rats treated according to the P, P+L and P+L+P protocols. Bar patterns: CON, empty; CP, black; DC, grey; DP, checked; DPC, horizontally striped.
Abscissa: treatment protocols. Ordinate: latency values, ms (mean + SD, n=10).
*$p<0.05$ vs. control group.

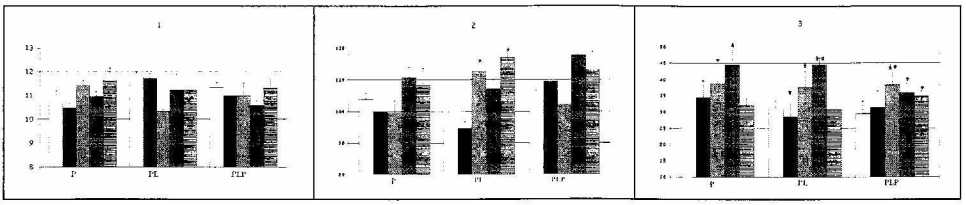

Figure 2: Duration of the cortical evoked potentials (1, somatosensory; 2, visual; 3, auditory) in control rats and in rats treated according to the P, P+L and P+L+P protocols. Bar patterns: CON, empty; CP, black; DC, grey; DP, checked; DPC, horizontally striped.
Abscissa: treatment protocols. Ordinate: latency values, ms (mean + SD, n=10).
*$p<0.05$ vs. control group.

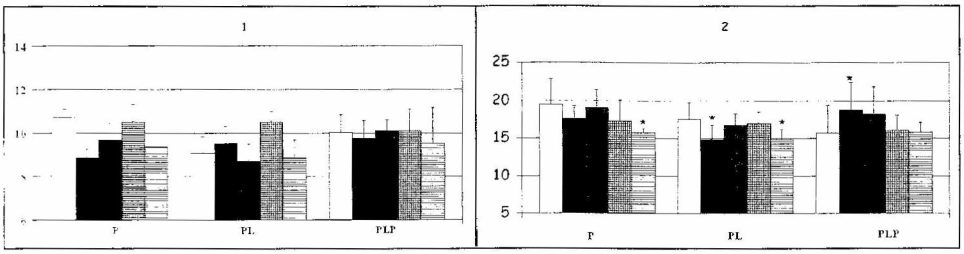

Figure 3: Relative refractory period (1) and conduction velocity (2) of the tail nerve, in control rats and in rats treated according to the P, P+L and P+L+P protocols. Bar patterns: CON, empty; CP, black; DC, grey; DP, checked; DPC, horizontally striped.
Abscissa: treatment protocols. Ordinate: latency values, ms (mean + SD, n=10).
*p<0.05 vs. control group.

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