Biochemical Pharmacology, Vol. 27, pp. 2647–2648.
Pergamon Press Ltd. 1978. Printed in Great Britain.

SHORT COMMUNICATIONS

Inhibition of acetylcholinesterase from foetal and maternal tissues after oral intake of Carbaryl (1-naphthyl-*N*-methyl-carbamate) by pregnant rats

(*Received* 4 *November* 1977; *accepted* 14 *March* 1978)

Carbaryl (Sevin), 1-naphthyl-*N*-methylcarbamate, is considered as the main representative of carbamate derivatives with anticholinesteratic activity, used as a broad spectrum insecticide.

The possible effects of Carbaryl upon reproduction, including teratology, have been investigated in a number of studies, with somewhat inconsistent results [1–4]. In the pregnant beagle dog [5] and in the guinea pig [6], Carbaryl produces teratogenic and toxic effects. In rhesus monkeys no terata were found but abortions were encountered [7]. We have already studied the placental transfer and the foetal accumulation of [^{14}C]-Carbaryl metabolites in mice and rats by autoradiography [8, 9, 10].

In this work we studied the anticholinesteratic activity of these metabolites upon the main organs of the foetus and then, in a dose–effect study, we tried to determine the lowest dose being efficient on acetylcholinesterase (AchE: E.C. 3. 1. 1. 7) of the foetus.

Nulliparous female Sprague–Dawley rats (220–240 g) were used. Copulation was ascertained by vaginal smears the morning after contact with a male. The finding of sperm was considered evidence of copulation and that day was recorded as day 1. Having received no food for 12 hr the animals are treated on the morning of the 18th day of gestation. They were then killed by decapitation and the organs removed and kept in ice.

Carbaryl obtained by crystallization in ethanol of technical grade Carbaryl (Rhône-Poulenc) was dissolved in pure maize oil (1%). Pregnant rats (8 animals) received only one dose (50 mg/kg) of Carbaryl and were killed 30 min, 1, 5, 12 or 24 hr after oral intubation.

Other animals are treated with decreasing doses of Carbaryl (50, 25, 12.5, 6.25 mg/kg) and then killed one hour later (8 animals per dose). Control animals received the same quantity of pure maize oil and were kept under the same conditions throughout the experiment.

Measurement of AchE activity. This measurement is carried out on haemolysed whole blood (1 vol. blood made up to 20 vol. water), on brain and liver homogenates obtained with 300 mg of tissue in 5.7 ml of 0.9% NaCl. All samples are maintained at 4°, the activity measurement being done the next day. The spectrophotometric method described by Ellmann [11] with acetylthiocholine iodide (5 m-mole/l in the test volume) as substrate and Phosphate buffer (50 m-mole/l, pH 7.2) was used; all AchE assays were carried out at 25°. This kinetic method is very suitable for measuring AchE activity in tissue samples inhibited by carbamates [12].

The protein content of all samples is determined by the colorimetric Biuret method [13].

A statistical study between mean values of treated animals and control animals is done by the *F* test from Fisher–Snedecor with a significance level of $P < 0.05$.

The differences in total protein concentration, between samples from control animals and treated animals in the two parts of the experiment (effect/time and effect/dose), are not statistically significant. The results are expressed in units of AchE/g protein for blood and in units of AchE/g tissue for the organs, brain and liver.

Table 1. The AchE activity of the foetal and maternal organs at different times after drug administration. The rats, 18 days pregnant, orally receive 50 mg/kg of Carbaryl at time zero. AchE activity of animals, killed 24 hr after treatment, is compared to control values of 19th day pregnant rats

	AchE units/g of protein		AchE units/g of organ			
	Maternal blood	Foetal blood	Maternal brain	Maternal liver	Foetal brain	Foetal liver
18th day Control	21.5 ± 3.5	20.9 ± 4.1	8.39 ± 1.9	3.74 ± 0.90	1.22 ± 0.17	1.45 ± 0.29
19th day	21.3 ± 3.9	16.7 ± 4.1	8.33 ± 1.6	3.56 ± 0.50	1.21 ± 0.04	1.22 ± 0.22
30 min.	13.1 ± 0.7 ‡	16.7 ± 1.9 *	2.76 ± 0.37 ‡	1.69 ± 0.30 ‡	0.98 ± 0.06 †	0.99 ± 0.14 ‡
1 hr	12.1 ± 1.3 ‡	15.1 ± 4.6 ‡	2.77 ± 0.7 ‡	2.01 ± 0.27 ‡	1.03 ± 0.09 *	0.99 ± 0.09 ‡
5 hr	13.3 ± 2.4 †	13.9 ± 2.9 ‡	3.13 ± 1.6 ‡	2.36 ± 0.88 *	0.99 ± 0.19 *	1.15 ± 0.27 *
12 hr	11.7 ± 1.9 ‡	8.7 ± 0.9 †	7.48 ± 1.9	2.34 ± 0.55 †	0.96 ± 0.11 ‡	1.06 ± 0.15
24 hr	18.5 ± 2.2	17.5 ± 4.5	7.70 ± 1.8	2.12 ± 0.56 ‡	1.12 ± 0.11	1.30 ± 0.18

Values are means ± S.E. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$.

Table 2. Effect of increasing doses of Carbaryl on the activity of the AchE of different maternal and foetal organs one hour after oral administration to 18 day-pregnant rats

	AchE units/g of protein		AchE units/g of organ			
Dose	Maternal blood	Foetal blood	Maternal brain	Maternal liver	Foetal brain	Foetal liver
Control	21.5 ± 3.5	20.9 ± 4.1	8.39 ± 1.9	3.74 ± 0.90	1.22 ± 0.17	1.45 ± 0.29
6.25 mg/kg	16.6 ± 2.1 †	16.2 ± 3.0 *	5.65 ± 1.5 †	3.55 ± 0.50	1.30 ± 0.13	1.48 ± 0.15
12.5 mg/kg	15.7 ± 3.1 †	12.5 ± 2.2 ‡	5.70 ± 1.8 †	3.67 ± 0.57	1.12 ± 0.07	1.35 ± 0.24
25 mg/kg	14.8 ± 2.4 ‡	13.3 ± 3.3 ‡	5.02 ± 1.8 †	3.05 ± 0.45	1.08 ± 0.09	1.41 ± 0.13
50 mg/kg	12.1 ± 1.3 ‡	15.1 ± 4.6 ‡	2.77 ± 0.7 ‡	2.01 ± 0.27	1.03 ± 0.09 *	0.99 ± 0.09 †

Values are means ± S.E. *P < 0.05, †P < 0.01, ‡P < 0.001.

The results of the study of enzyme activity with time are shown in Table 1 and the study of the dose/effect relationship in Table 2.

The animals were observed from drug administration onwards; we were thus able to note the rapidity (10 min) of the central and peripheral effects of Carbaryl which are due to its anticholinesterase properties.

Effect with time. Measurements of the activity of brain and blood AchE of the mother show a clear cut inhibition, brought about by Carbaryl or its metabolites, after 30 min, and that lasts about 12 hr.

The movement of Carbaryl or of its anticholinesterasic metabolites [14, 15] across the blood–brain barrier is fast. The inhibition of the hepatic AchE of the mother is long-term (24 hr).

The activity of blood, brain and liver AchE of the foetus drops markedly 30 min after the mother receives treatment. This inhibition can still be seen 12 hr after administration; it is significant in that it represents 20 per cent inhibition of the foetal brain AchE and over 25% for the blood and liver AchE.

Dose–effect relationship. Starting from a dose that is much less than the LD_{50}, doses were measured that did not bring about any significant inhibition of the foetal brain and liver AchE (Table 2). However, when the dose of 6.25 mg/kg is reached, Carbaryl causes a 20 per cent reduction of the AchE activity of the foetal blood. This stresses the high sensitivity of the foetus to this drug. However, even though inhibition of the foetal blood AchE is brought about from the very lowest dose used, the foetal brain and liver AchE is only inhibited with large doses. The blood–brain transfer in the foetus would thus seem different to that of the mother. This inhibition of the maternal brain AchE confirms the visual observations of the toxic effect on the central nervous system seen from the lowest dose (6.25 mg/kg).

The work has allowed us to show the foetal toxicity limits as well as the anticholinesterase activity of Carbaryl in the different maternal and foetal tissues. The AchE of the foetus are sensitive to weak doses of Carbaryl shortly after administration of the drug to the mother. The effect persists 12 hr after a single treatment.

Work being undergone at present should give details on chronic treatment of foetus and new-born rats and also help in the determination, *'in vitro'*, of the inhibition constants of the foetal AchE as well as in shedding light on the interaction between foetal proteins and Carbaryl.

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