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Inhibition of serine esterases in different rat tissues following inhalation of soman

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The acute toxic effects of organophosphates (OPs) are due to inhibition of acetylcholinesterase (AChE) (EC 3.1.1.7). The inhibition of other serine esterases, such as butyrylcholinesterase (BuChE) (EC 3.1.1.8) and carboxylesterase (CarbE) (EC 3.1.1.1) does not induce any known physiological alterations. Recently, BuChE has been shown to coregulate acetylcholine lifetime in canine trachealis muscle and may therefore in some tissues play a role in the breakdown of acetylcholine (ACh) [1]. BuChE and especially CarbE may, however, be important for detoxification of low doses of OPs in rodents, since they have a high plasma concentration of CarbE [2–4].

A good correlation between the concentration of plasma CarbE and the LD_{50} of the OP soman in the developing rat has previously been shown [5]. Furthermore, injection of partially purified rat liver CarbE into 14-day-old rats increased the tolerance to soman, indicating that CarbE in plasma may be of great importance for the detoxification of organophosphorus compounds. CarbE may thereby function as a very important barrier which limits the distribution of the toxic agent to vital organs [6], since the difference in plasma concentration of CarbE between different species correlates with the difference in LD_{50} [7].

The aim of the present work was to elucidate whether CarbE in respiratory tissue and plasma plays an important role in detoxification of soman during inhalation exposure.

Materials and Methods

Chemicals. [1-^{14}C]Acetylcholine chloride ([^{14}C]ACh) was purchased from Amersham International (Bucks, U.K.). Ethopropazine (10-[2-(diethylamino) propyl]-phenothiazine) and 4-nitrophenyl butyrate were from the Sigma Chemical Co. (Poole, U.K.). Soman (*O*-[1,2,2-trimethyl-propyl]-methyl-phosphonofluoridate), assessed to be more than 99% pure by nuclear magnetic resonance spectroscopy, was synthesized in our laboratory. All other chemicals were of analytical laboratory reagent grade.

Inhalation method. Whole body exposures of male Wistar rats (200–300 g) (Møllegard, Copenhagen) to sub-acute concentrations of the acetylcholinesterase inhibitor soman were carried out in a dynamic inhalation system designed specifically for the exposure of small rodents to highly toxic gases [8]. Two rats were exposed simultaneously in a glass chamber of 2200 mL, the atmospheric concentration of soman was measured by gas chromatography (Carlo Erba, HRGC 5160) with a nitrogen/phosphorus detector.

No symptoms of poisoning were observed during the inhalation period.

Enzyme activity assays. The total cholinesterase (ChE) activity was determined by the radiochemical method of Sterri and Fonnum [9] at 30°. The AChE activity was measured after inhibition of BuChE by 0.2 mM ethopropazine [10]. The CarbE activity was measured by the spectrophotometric method of Ljungquist and Augustinsson [11] with modifications [12]. The protein concentration was determined by the method of Lowry *et al.* [13].

Means and standard error of the mean (SEM) were calculated for all data. The Student's *t*-test was used to assess the significance of the differences between data groups.

Results and Discussion

The airways and lungs are the first tissues exposed to toxic gases and vapours and are also the primary uptake sites for some OPs. The results from this study show that long-term exposure to low concentrations of soman primarily inhibits the cholinesterases of the respiratory tissue, plasma and erythrocytes and the CarbE of plasma and airways (Table 1). Although there were no symptoms

Table 1. Activity of AChE, BuChE and CarbE in different tissues in the rat following 40 hr inhalation exposure to two different concentrations of the organophosphorus anticholinesterase soman

Enzyme	Ct (soman) ± SEM (N = 6) (mg min/m³)	Mean per cent enzyme activity ± SEM					
		Airways	Lung	Diaphragm	Brain	Plasma	Erythrocyte
AChE	0	100 ± 9 (8.82) N = 14	100 ± 8 (3.17) N = 14	100 ± 13 (3.83) N = 14	100 ± 11 (49.2) N = 7	—	100 ± 10 (1.95) N = 12
	128 ± 25	15 ± 3 *** N = 12	17 ± 3 *** N = 12	95 ± 14 NS N = 12	123 ± 17 NS N = 8	—	15 ± 3 *** N = 12
	560 ± 77	2 ± 1 *** N = 9	5 ± 2 *** N = 9	26 ± 7 *** N = 9	59 ± 12 * N = 7	—	8 ± 3 *** N = 7
BuChE	0	100 ± 11 (17.5) N = 14	100 ± 8 (4.78) N = 14	100 ± 17 (1.55) N = 14	100 ± 22 (8.45) N = 7	100 ± 15 (1.34) N = 14	—
	128 ± 25	7 ± 3 *** N = 12	37 ± 5 *** N = 12	31 ± 12 ** N = 12	50 ± 18 NS N = 8	31 ± 9 *** N = 12	—
	560 ± 77	0 *** N = 9	8 ± 3 *** N = 9	41 ± 11 * N = 9	65 ± 23 NS N = 7	8 ± 3 *** N = 9	—
CarbE	0	100 ± 15 (280) N = 14	100 ± 10 (431) N = 14	100 ± 22 (102) N = 14	100 ± 9 (60.2) N = 7	100 ± 8 (81.8) N = 14	—
	128 ± 25	53 ± 12 * N = 12	63 ± 5 ** N = 12	81 ± 12 NS N = 12	97 ± 9 NS N = 8	39 ± 6 *** N = 12	—
	560 ± 77	25 ± 5 *** N = 9	69 ± 8 * N = 9	59 ± 12 NS N = 9	102 ± 7 NS N = 7	12 ± 2 *** N = 9	—

Activities of acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and carboxylesterase (CarbE) in per cent of control (unexposed rats) after exposure to 0.05 and 0.2 mg/m³ (40 hr) of soman. Specific activity (nmol/min/mg protein) is given in parentheses for each control. The enzyme activities were determined as described in Materials and Methods. Values represent mean ± SEM of N animals.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P \geq 0.05$.

of poisoning observed during the inhalation experiments, also the cholinesterases in the diaphragm were inhibited to a large extent by soman.

The inhalation experiments (40 hr) with two concentrations of soman (128 ± 12 and 560 ± 77 mg min/m^3) inhibited the AChE activity in the airways (85 and 98%, respectively), lung (83 and 95%, respectively) and erythrocytes (85 and 92%, respectively) to approximately the same extent. The BuChE activity in lung (63 and 92%, respectively) and plasma (69 and 92%, respectively) were inhibited to approximately the same extent after the soman exposure, although BuChE was apparently less inhibited than AChE. Inhibition of AChE and BuChE were less pronounced in the diaphragm and the brain at both concentrations of soman than in the other tissues examined. This difference in the degree of inhibition is probably due to detoxification by covalent binding of soman to the active site of CarbE and BuChE before soman actually reaches these target tissues [2, 3, 4, 14, 15]. Furthermore, hydrolysis of soman in the liver by phosphorylphosphatases limits its accumulation in the blood [16].

The CarbE activities in diaphragm and brain were not significantly inhibited by soman. The CarbE activity in plasma, however, was significantly ($P < 0.001$) inhibited (61 and 88%, respectively) following exposure to both the high and the low concentration of soman as were the CarbE activities in airways (47 and 75%, respectively) and lung (37 and 31%, respectively). There are two main groups of CarbE in plasma that can be separated on the basis of their specificity to methyl butyrate and 4-nitrophenyl butyrate [12]. It has been suggested that the plasma CarbE with the highest specificity for 4-nitrophenyl butyrate is the most important enzyme for detoxification after injection of soman [5, 6, 12].

Our results show that inhaled soman inhibits plasma CarbE significantly more than the CarbE in lung and airways. Separate studies have shown that there is only a small difference in the bimolecular inhibition constants of plasma and lung CarbE with soman (R. Gaustad, NDRE, personal communication). Since the cholinesterases of the respiratory tissues, including lung, and the blood were equally inhibited, the small reduction of the CarbE activity in lung compared to plasma was surprising. One possible explanation is the cellular localization of the enzymes. It may be that lung CarbE is not readily available to soman. A similar difference in the level of inhibition between ChE and CarbE is seen in rat liver after *in vitro* perfusion with soman [17].

In summary, the potent inhibition of CarbE activity in plasma during long-term (40 hr) sub-acute inhalation exposure to soman indicates that CarbE in plasma represents a very important barrier to sub-acute concentrations of inhaled soman. The CarbEs in the airways and the lung are inhibited to a lower extent and thus seems not to be as important as plasma CarbE. The CarbEs in the respiratory tissue are, however, more inhibited after inhalation exposure compared to after injection of soman [12], and may accordingly play a more important role in the detoxification of inhaled soman.

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* Correspondence to: P. Walday, Norwegian Defence Research Establishment, Division for Environmental Toxicology, PO Box 25, N-2007 Kjeller, Norway.

Norwegian Defence Research Establishment
Division for Environmental Toxicology
2007 *Kjeller*
Norway

PER WALDAY*
PÅL AAS
FRODE FONNUM

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