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EFFECT OF SOMAN ADMINISTRATION ON β-ESTERASES IN BLOOD, LIVER MICROSOMES AND BRAIN REGIONS OF RATS

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Abstract: Activities of enzymes cholinesterase (ChE) and carboxylesterase (CaE) were assayed in serum, liver microsomes and three regions of brain, viz; cerebrum, cerebellum and brain stem (with mid brain) in male albino rats at 0.5 and 2 h periods after administration of 1/2 LD 50 dose of soman (0.22 mg/kg) intraperitoneally in olive oil as vehicle. At 0.5 h, in serum, ChE activity declined to 33% of its initial level whereas CaE activity was almost completely inhibited. However, in the liver microsomes at this period, ChE activity was greatly inhibited (18% of initial level) whereas CaE activity was nearly unaffected. At 2 h period, both the enzymes in the serum were almost completely inhibited. In the brain regions (excepting in cerebellum), both the enzymes were nearly similarly inhibited (by 55% to 65% of the initial level) at both the periods. The time related differential response of these two β–esterases in acute soman intoxication probably occured in the peripheral tissues like blood and liver but not in the CNS.

Key words: serum, carboxylesterase, cholinesterase, rats, brain regions, liver microsomes, soman

INTRODUCTION

Soman (pinacolyl methyl phosphonofluoridate) is an organophosphorus compound (OPC) which is highly toxic and is therefore a potent chemical warfare agent. Its toxic action is ascribed to its ability to irreversibly inhibit one of the β–esterase (1) acetylcholinesterase (AChE) by phosphorylation of serine hydroxyl group at its active site (2). As a result, the enzyme is unable to catalyze the hydrolysis of neurotransmitter acetylcholine which therefore accumulates at neuromuscular junctions and at several other structures in the central nervous system, leading to impairment of many physiological functions, and eventual death. Apart from binding to AChE which is probably the main cause of its toxicity (3), soman also binds to other targets in the body of the animals (4). Several investigators have suggested that these alternative 'non–cholinesterase' soman binding sites may be beneficial in soman poisoning (5). The ensyme carboxylesterase (CaE) which is another β–esterase type of enzyme (1) is considered an important 'non-cholinesterase' soman binding site in the tissues (6). Its main function in the body is to hydrolyze short chain fatty acids and its structural analogs, but has no other known physiological function (7). It has been reported that pretreatment of animals with CBDP (2–(0–cresyl) 4 H–1:3:2–benzodioxa) phosphorin–2–oxide) and TOCP (triortho cresyl phosphate) which bind at the active site of CaE and thereby inhibit its catalytic activity, potentiate soman toxicity (8) whereas pretreatment with phenobarbital which increases CaE synthesis by its induction (9) lowers soman toxicity (10), indicating that the beneficial effect of CaE is probably due to its scavenging of soman. CaE is ubiquitously present in the body and is largely concentrated in the blood plasma and in liver, in the later a very high concentration of CBDP sensitive soman binding sites have been demonstrated (11). Although there are several reports in the literature on effect of OPC on AChE activity in brain regions (12,13) there are few studies, if any, on CaE of the brain on OPC intoxication. The present study is aimed in that direction.

METHODS

Animals: Experiments were performed on male wistar rats weighing 100 ± 5 g. Drinking water and commercial rat diet were freely available to the

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animals *ad libitum*. All animals were maintained on a 12 h day and night cycle. They were fasted 18 h before use in the experiments. The LD 50 of soman in these animals was ascertained as desribed previously (10) and was observed to be 0.44 mg/kg intraperitoneally (ip). 1/2 LD50 dose of soman (0.22 mg/kg, ip) was administered as a single dose in olive oil as vehicle. The amount of vehicle used for injections was always less than 0.5% of the body weight of the animals. Controls were sham injected with vehicle only. At the end of 30 min period after injection, the animals were killed by decapitation and the blood from the excised necks was collected in heparinized tubes. It was centrifuged at 3000 rpm for 10 min to separate the plasma in which ChE and CaE activities were assayed immediately. Livers were dissected out freed of any adhering blood by adsorption on filter papers and immediately stored at –20°C for preparation of microsomes on the next day. Brain was dissected out immediately into three major brain areas by the method of Glowinski (14), viz; cerebrum, cerebellum and brain stem with mid brain, which comprised of medulla oblongata, pons, mid brain and striatum. The separated brain regions were immediately weighed on a torsion balance and suspended in ice cold 0.32 M sucrose containing Triton–X 100. A 10% w/v homogenate of tissue was prepared in this medium in an all glass Potter-Elvhjem homogenizer using 5 to 6 strokes with hand. Triton X–100 was used in the homogenization medium in order to solubilize the membrane bound AChE and CaE enzymes (15) in order to assay the activity of total, viz; soluble plus membrane bound esterases, because it is known that soluble esterases comprise a maximum of only 20% of the total AChE present in the brain (16). AChE activity was assayed by the method of Ellman et al (17) and CaE by the method of Kaneko et al (18). Protein was determined by the standard method of Lowry et al (19).

Chemicals: All chemicals were of analytical grade from Sigma Chemicals (St Louis, Mo. USA). Soman was synthesized 99% pure at the synthetic chemistry division of this establishment and purity was checked by IR, NMR and GC sepctra.

RESULTS AND DISCUSSION

Results in Table I on effect of soman administration (1/2 LD50, ip) after 1/2 h show that in this period serum ChE activity declined to 33% of its initial value whereas serum CaE was almost completely inhibited (4% of its initial value). The inhibition pat-

TABLE I : Effect of soman on β-esterases in blood serum, liver microsomes and brain regions of rats.

	Cholinesterase			*Carboxylesterase*		
	Control	*Soman treated*		*Control*	*Soman treated*	
		1/2 h	*2 h*		*1/2 h*	*2 h*
Serum	0.70±0.03	0.233±0.03* (33)	Nil	10.4±0.77	0.43±0.06* (4)	0.61±0.02* (6)
Liver microsomes	22.0±0.45	4.12±0.29* (18)	—	3213±271	3011±241.0 (93)	—
Cerebrum	110.8±6.7	62.3±5.7* (56)	53.6±3.4* (48)	99.4±4.5	66.0±5.6* (66)	67.0±5.60* (67)
Cerebellum	49.6±3.4	46.1±3.8 (94)	28.3±4.1* (55)	66.2±5.0	61.9±4.4 (93)	40.6±6.30* (62)
Brain stem with mid-brain	193.3±9.4	103.9±10.6* (53)	89.2±5.3* (46)	94.9±5.4	59.0±5.2* (62)	65.7±3.90* (69)

1. Values are Mean ± SEM. Values in parenthesis are percent activity of control.
2. Cholinesterase activity is μ mol thiocholine/min/ml serum/g protein in brain regions/liver microsomes at 37°C
3. Carboxylesterase activity is μ mol a-napthol/min/ml serum/g protein in brain regions/liver microsomes at 37°C
4. * = $P < 0.01$

tern of these two enzymes was reversed in the liver microsomes at this period as liver CaE activity was statistically unchanged (P> 0.05) whereas ChE activity was highly inhibited and was only 18% of its inital value. Unlike serum and liver, in the brain regions both the esterases were almost similarly inhibited on soman administration to 60±5% levels in the cerebrum and brain stem with mid brain (but not the cerebellum) at 1/2 h. At 2h after soman administration, CaE in the serum remained as much inhibited as at 1/2 h period, whereas serum ChE activity further declined and was totally inhibited as compared to its 33% inhibition at 1/2 h period. In the brain regions, the degree of inhibition of these two enzymes at 2 h period was almost similar to that observed in the $\frac{1}{2}$ h period as the enzyme activities were 50±5% (ChE) and 65±5% (CaE) excepting in the cerebellum in which these two enzymes were not inhibited at $\frac{1}{2}$ h period but were inhibited at 2 h period and were about 55-62% of their initial values.

It is assumed that regional approach to the study of acute OPC intoxication may provide more detailed information about the changes in AChE activity than the study on whole brain (16). However, many investigators have observed contrary results, viz; no differences in the inhibition of AChE in the various brain regions after the administration of OPC like DFP and soman (20, 21). It has been postulated that the observed difference by several workers in AChE activity in different brain regions during different periods of study (varying between several hours to days) could be due to the difference in the de novo synthesis and/or spontaneous reactivation of the enzymes in various regions at these different periods (22). The results in Table I shows that AChE was almost similarly inhibited (50 ± 5% activity) in all the three studied brain regions in 2 h which supports the observations of several workers (20, 21). However, in the earlier period of 1/2 h after soman, the enzyme was not inhibited in the cerebellum contrary to its inhibition in the other two brain regions which had 53 to 56% activity of the enzyme (Table I). Since spontaneous reactivation or de novo synthesis of enzyme in the 1/2 h period is unlikely, non-inhibition of AChE in cerebellum at this period could be due to the insufficient amount of inhibitor transported through blood to this brain region within this short period as it is known that blood flow to brain follows regional differences depending on the metabolic status of the region (23) and cerebellum is metabollically less active and has lower rate of blood flow than the other brain regions (23). It may be argued that the difference in the affinity of the inhibitor (soman) to various AChE molecular forms (AChE isozymes), which may be present in different proportion in the various brain regions (24) too may lead to varying degree of AChE inhibition as observed by previous workers (16). However, results in Table I show that another soman sensitive esterase, CaE also showed almost similar pattern of inhibition as AChE at both 1/2 and 2 h periods in these brain regions (Table I) which tends to support the former assumption that inhibitor concentration more likely was the limiting factor in differential inhibition of various brain regions than the sensitivity of various AChE isozymes to the inhibitor, because it is unlikely that both the esterases would have similar soman sensitive isozymes with similar quantitative distribution in the studied brain regions. Although CaE does not have any role in the neurotransmission (7), it is assumed that it is capable of diminishing soman toxicity by scavenging it, viz; by binding it so that its availability in the free form is decreased which spares the enzyme AChE from inhibition and brings down the lethality of soman (4–6). It is reported that plasma CaE may be more important than liver CaE in protection against soman due to greater binding affinity of former as compared to the later for soman (10). This assumption is supported by the results in Table I which show that in serum, CaE enzyme was more inhibited (96% inhibition) accompanied by lesser inhibition of ChE (67% inhibition), whereas in the liver, ChE enzyme was more inhibited (82% inhibition) accompanied by very low inhibition of CaE (7% inhibition). However CaE in various brain regions was nearly as much inhibited as AChE by the administered soman dose, in a nearly parallel manner with minor differences in various regions as described above and it used up nearly half of the inhibiting ability of soman, showing that brain CaE was as important as blood CaE in protection against soman toxicity. This assumption might explain some intriguing observations in soman intoxication like several fold increased toxicity of 0.5 LD50 dose of soman by pretreatment with CaE inhibitor CBDP, by a dose that was too low to have any effect on liver CaE activity and could only slightly lower the lung and blood CaE activity (6), because increased lethality

of soman in this condition may be ascribed to the blocking of soman binding sites of brain CaE by CBDP. These results show the important role of CaE of brain in addition to well known role of CaE of peripheral tissues in protection against soman.

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