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Organophosphate-mediated inhibition of choline acetyltransferase activity in rat brain tissue[1]

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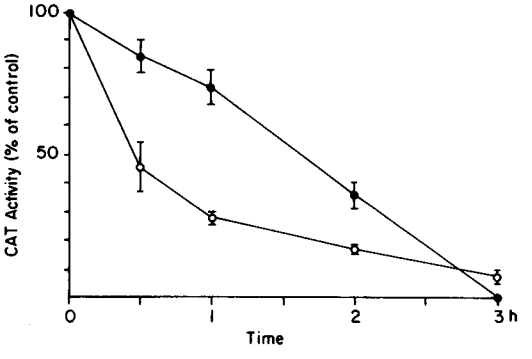
Summary. Administration of the organophosphate compound soman in rats resulted in an inhibition of choline acetyltransferase activity in almost all brain regions examined. Enzyme activity was inhibited by 20–50% in various brain regions 30 min after soman injection (94–120 µg/kg). Enzyme activity in two regions decreased with time to a near zero level by 3 h after injection.
Key words. Choline acetyltransferase; organophosphate; soman; acetylcholinesterase; enzyme inhibition.

The organophosphate compounds appear to exert their acute toxic effects by inhibiting cholinesterase enzymes throughout the body[3]. These enzymes include the cholinesterases of plasma (acylcholine acyl-hydrolase, ChE, E.C. 3.1.1.8) and various tissues, particularly the acetylcholine acetyl-hydrolase (AChE, E.C. 3.1.1.7) in nervous tissue and at the neuromuscular junction. Lethality associated with organophosphate intoxication usually results from accumulation of acetylcholine (ACh). The accumulation of ACh can cause respiratory failure as a consequence of synaptic blockade at the diaphragm neuromuscular junction or in CNS respiratory centers. While the mechanism underlying the acute effects of organophosphates is known, intoxication with these compounds can also produce long-lasting psychiatric effects[4–6]. The mechanism by which these persistent psychiatric changes are produced is uncertain.

Soman (methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester) is a highly toxic organophosphate compound[7–10]. Soman exhibits most of the general features of the organophosphate group, it is a potent inhibitor of AChE and has an LD_{50} in rats in the range of 80–100 µg/kg[8]. The work described in this report represents an investigation of one particular type of potential organophosphate-induced secondary effect. This secondary effect involves presynaptic alterations in the activity of the synthetic enzyme of ACh, choline O-acetyltransferase (CAT, E.C. 2.3.1.6), after soman administration. Such an effect may reveal the basis for long term psychiatric changes caused by organophosphates. For this reason, mechanisms of action of the organophosphates other than AChE inhibition are of interest.

Methods. Sprague-Dawley rats (200–300 g) were used in all experiments. Rats were administered soman (94–120 µg/kg) by s.c. injection. The soman was administered in physiological saline with an injection volume of 0.2 ml. Control animals were given saline injections. Animals which did not survive were not used. All animals surviving were sacrificed by decapitation at a given time interval after injection. Whole brains were removed and the indicated areas of tissue were manually dissected. Tissue was homogenized in buffered saline and CAT activity was assayed according to the radiometric procedure of Fonnum[11]. The activity of AChE was also determined in the different brain areas from the same animals. The assay used to determine AChE was that described by Reed et al.[12]. In this way, CAT activity could



Time-dependent inhibition of CAT activity by soman in two different areas of brain tissue. All animals were injected with 94 µg/kg of soman and sacrificed at the indicated time intervals thereafter. Enzyme activity in the basal forebrain (○) and cerebellum (●) was then determined. The 100% value at time 0 corresponds to the control level of CAT activity in each tissue.

be correlated with the level of AChE inhibition. Protein determinations were performed with the Bradford assay procedure[13].

Results. A single dose of soman produced marked effects on brain CAT activity. As shown in the table, CAT activity was decreased in almost all the brain regions examined. These decreases were well below the control levels. The only areas which showed no reduction in CAT were frontal cortex and mesencephalon. In all the other areas, CAT enzyme activity was inhibited by 20–50%. An examination of AChE activity revealed that this soman dosage produced greater than 85% inhibition of the enzyme in all ten brain regions (data not shown). No real differential sensitivity of AChE to soman was detected in any of the brain areas.

Alterations in CAT activity as a consequence of AChE inhibition by organophosphates has not been previously reported. Therefore, at this point the mechanism leading to changes in CAT enzyme activity is unknown. In an effort to elucidate a possible interaction between CAT activity and AChE inhibition, CAT activity was monitored at different time intervals after soman injection. As shown in the figure, CAT activity exhibited an overall pattern of decrease with time in basal forebrain and cerebellum. By 3 h after soman administration, there was little or no CAT activity remaining in these two regions. While AChE activity was almost totally inhibited in basal forebrain and cerebellum at 30 min postinjection, an overall pattern of slow recovery of AChE activity was observed over the ensuing 2–3 h. By 3 h after injection, AChE activity in the basal forebrain had achieved a value of 6.4% of the previously determined control level in this region.

Discussion. The results of this study indicate an effect of soman on ACh synthesis via reduction in the level of CAT enzyme activity. While the exact mechanism underlying the effect on CAT activity is uncertain, it seems quite likely that this may represent a secondary effect of AChE inhibition. The initial alteration in CAT activity occurs almost simultaneously with AChE inhibition. One plausible explanation for the present findings is that soman causes a severe depletion of intraterminal ACh. This occurs as ACh is not hydrolyzed in the synaptic cleft due to AChE inhibition. Thus, no choline is provided for further ACh synthesis in the terminal and since ACh itself cannot be reclaimed, there is a depletion of ACh within the terminal. The relatively complete loss of ACh causes a loss of functionality and/or death of the terminal. The death of the terminal produces the decrease or loss of CAT activity. This mechanism would be consistent with the loss of cholinergic terminals suggested in the delayed neuropathy described by other investigators[6], although the timeframe is certainly different. While this hypothetical mechanism provides a reasonable explanation for the results obtained in this study, at present it certainly represents speculation.

In conclusion, an inhibition of CAT activity by the organophosphate soman has been determined. This effect is considered to be a presynaptic secondary effect of AChE inhibition; however, the mechanism is unknown.

Soman-mediated inhibition of CAT in different brain regions[a]

Brain region	Control[b]	Soman-treated[c]	% inhibition
Frontal cortex	23.3 ± 2.2	35.3 ± 4.2	0.0
Occipital cortex	37.6 ± 7.2	19.9 ± 3.6	47.1
Parietal cortex	28.0 ± 6.4	12.9 ± 2.3	53.9
Temporal cortex	31.9 ± 2.3	24.0 ± 1.9	24.8
Hippocampus	50.2 ± 7.2	37.2 ± 3.2	25.9
Basal forebrain	100	48.3 ± 8.1	51.7
Cerebellum	5.3 ± 2.4	4.1 ± 0.6	22.6
Mesencephalon	44.5 ± 8.1	45.2 ± 6.3	0.0
Thalamus/hypothalamus	31.7 ± 0.7	22.0 ± 2.1	30.6
Corpus striatum	95.3 ± 4.7	58.8 ± 8.3	38.3

[a] All values for CAT activity are expressed as a percentage of the level in the basal forebrain control, the level of activity in the basal forebrain control equals 100% by definition. [b] All values are the mean ± SD of triplicate determinations. [c] The soman dose ranged from 94 to 120 µg/kg. All animals were sacrificed 30 min after injection.

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Distribution of (Na^++K^+)-ATPase in the hindgut of *Glossina morsitans morsitans* Westwood

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Summary. (Na^++K^+)-ATPase activity was higher in preparations from the ileum of *Glossina mortisans* than in those from the rectum. This result suggests that the ileum, as well as the rectum, may play a role in osmoregulation in the tsetse fly.

Key words. Glossina morsitans; hindgut; (Na^++K^+)-ATPase.

The distribution of (Na^++K^+)-ATPase activity in the alimentary canal of *Glossina morsitans morsitans* Westwood has been described previously[1] and it was shown that (Na^++K^+)-ATPase activity was highest in the anterior midgut and lowest in the posterior region of this organ. The (Na^++K^+)-ATPase activity of the hindgut (ileum and rectum combined) was intermediate. The present paper describes the distribution of (Na^++K^+)-ATPase activity in the hindgut of *G.m.morsitans* and shows