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Acute exposure to a low or mild dose of soman: Biochemical, behavioral and histopathological effects

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Abstract

Effects of low to mild doses of soman on central and blood cholinesterase (ChE) activities and anxiety behavior were studied in mice 30 min, 24 h and 7 days after poisoning. At these two latter time points, histopathological consequences of soman intoxication were also studied. The 30-μg/kg dose of soman produced 30 min after intoxication, about 35% of central ChE inhibition, and an anxiolytic effect without toxic signs or histopathological changes. The 50-μg/kg dose of soman produced at the same time, about 56% of central ChE inhibition, slight clinical signs of poisoning without convulsions, an anxiogenic effect with a slight hypolocomotion but no brain damage. A mild dose of soman (90 μg/kg) produced at this same time point about 80% of central ChE inhibition, and led to ataxia and tremors in every mouse and to convulsions in some of them. Thirty minutes and 24 h after poisoning, the behavioral tests revealed neither anxiolytic nor anxiogenic responses despite a clear hypolocomotion. Only mice that experienced long-lasting convulsions developed neuropathological changes. The functional implication of our results, as well as the biological relevance of blood vs. brain ChE levels, as an index of intoxication severity are discussed.

Keywords: Anxiety; Acute soman exposure; Cholinesterase; Elevated plus-maze; Histopathology; TUNEL; Mice

1. Introduction

Soman (*O*-1,2,2-trimethylpropylmethylphosphonofluoridate) is a potent and irreversible organophosphate (OP) inhibitor of both peripheral and central cholinesterases (ChE). It is well known that an acute administration of soman at doses close or superior to the LD_{50} induces generalized convulsive seizures and subsequent encephalopathy (Lemercier et al., 1983; McLeod et al., 1984). While the toxic effects induced by a convulsant dose of soman are well described (Brown and Brix, 1998; McDonough and Shih, 1997; Shih and Scremin, 1992), notably in primates (Lallement et al., 1999), there are only few and sometimes contradictory data on the acute effects of nonconvulsant doses (see reviews in Brown and Brix, 1998; Moore, 1998; Ray, 1998). For example, whether or not subconvulsant doses of soman can produce neuropathology still remains questionable (Goldman et al., 1993; Kadar et al., 1992; McDonough and Shih, 1997; Petras, 1994). Moreover, a confusion may exist about the exact definition of a low-level vs. a high-level exposure to OP nerve agents (Brown and Brix, 1998; Moore, 1998; Ray, 1998) depending on the considered criteria (overt signs of toxicity, ChE activity level, etc.). At last, since various protocols of intoxication with low doses of OP (e.g., repetitive or acute administration) have been used, analysis of the results from the available literature is rather difficult. Obviously, a single low dose of soman does not produce the same effect as a low dose repetitively administered either over a short period of time (cumulated toxicity is expected) or at longer intervals (down-regulation of cholinergic system is expected) (e.g., as reviewed in Brown and Brix, 1998). Thus, it is of paramount importance to clarify whether nonconvulsant doses of OP compounds are able or not to produce more or less subtle neurobiological changes including neuropathological and behavioral sequelae. This knowl-

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edge might be of a particular interest in the still open debate on the implication of low OP doses in the Gulf War syndrome (e.g., Kurt, 1998) and in the analysis of the long-term consequences of the exposure to sarin consecutive to the terrorist attacks against Japanese civilians in 1994 and 1995 (Yokoyama et al., 1998).

A previous study showed that acute nontoxic doses of soman affect the anxiety behavior of rats (Sirkka et al., 1990). However, no relationships between this behavioral change, inhibition of central or blood ChE and histopathological sequelae have been deduced. The objective of the present investigation is to examine in mice the combined effects of soman on behavioral anxiety, central and blood ChE inhibitions and histopathological sequelae up to 7 days after intoxication. Observations are performed 30 min, 24 h and 7 days after soman poisoning. Three infra-lethal doses of soman are adopted according to clinical observations: the first one (30 μg/kg, 0.27 LD_{50}) corresponds to an exposure that does not result in clinical signs or symptoms; the second (50 μg/kg, 0.45 LD_{50}) leads only to slight clinical signs (weak hypolocomotion, transient and weak tremors); and the last one (90 μg/kg, 0.82 LD_{50}) corresponds to the threshold dose that may lead to convulsions in some of the animals.

2. Methods

Principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were followed in all experiments. The experimental designs of the studies reported herein were approved by the local Ethics Committee of our Institute.

2.1. Animals and soman intoxication

Male Swiss mice weighing 30–33 g (Elevage Janvier, France) were maintained in cages (10 per cage) with food and water ad libitum in a light-controlled room (light on from 7:00 a.m. to 7:00 p.m.) at controlled temperature. All behavioral tests were conducted during the light portion of the cycle (between 8:00 and 11:30 a.m.) to minimize the effects of circadian variations on cholinergic parameters (Elsmore, 1981; Hanin et al., 1970; Saito et al., 1975). Mice were injected with soman (30, 50 or 90 μg/kg sc in normal saline in an injection volume of 200 μl) or saline for control.

2.2. ChE activity study

To follow the inhibition of both peripheral and central ChE during the time course of an intoxication by either 30, 50 or 90 μg/kg of soman, animals were distributed in 19 groups ($n = 8–11$ per group) as listed in Tables 1 and 2. The controls received normal saline instead of soman. Whole blood samples were obtained by retroocular sampling with a heparinized capillary, then immediately diluted (1/10) with 1% saponin, frozen in liquid nitrogen and stored at −80°C until measurement (Wetherell, 1994) that was performed according to the method of Ellman et al. (1961) with automated procedure (Hitachi 704, Kit Reagent MPR2 124117, Roche Molecular Biochemical). The baseline level of blood ChE was obtained on a sample drawn 30 min before soman intoxication. Then, blood ChE activity was measured 30 min, 24 h or 7 days after intoxication. After the blood sampling at 30 min, 24 h or 7 days post-soman, the mice were sacrificed by decapitation for the central ChE measurement. The brains were quickly removed and frozen at −20°C until measurement. The brains were homogenized in 50 mM Tris buffer, pH 7.4, 0.32 M saccharose. After centrifugation for 15 min at $1000 \times g$, ChE were measured on supernatants as described above.

2.3. Histopathology

In a different experiment, 86 mice were injected with 30, 50 or 90 μg/kg of soman and sacrificed 24 h or 7 days after intoxication (10–19 mice per group). Ten supplementary animals were administered with saline instead of soman and served as controls. For each animal, the blood ChE activity was measured to assess the severity of soman exposure and the inhibition level was verified by comparing the results

Table 1
Peripheral cholinesterase measurements (whole blood)

Cholinesterase whole blood	Delay after intoxication	Cholinesterase (baseline) (nmol/min/l)	Cholinesterase (nmol/min/l)	Cholinesterase (% of inhibition)
Soman (30 μg/kg)	30 min	4216 ± 220 ($n = 10$)	2359 ± 140 ($n = 10$)**	43.9
	24 h	4064 ± 160 ($n = 8$)	3401 ± 139 ($n = 8$)*	16.3
	7 days	4220 ± 85 ($n = 11$)	4011 ± 93 ($n = 11$)*	4.9
Soman (50 μg/kg)	30 min	4101 ± 122 ($n = 10$)	1498 ± 85 ($n = 10$)**	63.3
	24 h	3927 ± 114 ($n = 10$)	2634 ± 77 ($n = 10$)**	32.6
	7 days	3972 ± 139 ($n = 11$)	4189 ± 134 ($n = 11$)	− 7.8
Soman (90 μg/kg)	30 min	3846 ± 205 ($n = 10$)	545 ± 46 ($n = 10$)**	85.9
	24 h	4273 ± 105 ($n = 10$)	2645 ± 79 ($n = 10$)**	37.9
	7 days	4390 ± 143 ($n = 9$)	4077 ± 159 ($n = 9$)*	7.1

Values represent the mean ± S.E.M. Statistical analysis was performed with Wilcoxon's test for dependent samples.

* $P < .05$.

** $P < .01$.

Table 2
Central cholinesterase measurements

Central cholinesterase	Delay after intoxication	Number of animals	Cholinesterase (nmol/min/mg proteins)	Cholinesterase (% of inhibition)
Controls		10	65.8 ± 1.23	
Soman (30 μg/kg)	30 min	10	42.97 ± 1.23*	34.7
	24 h	10	50.31 ± 0.57*	23.5
	7 days	11	55.61 ± 0.67*	15.5
Soman (50 μg/kg)	30 min	10	28.72 ± 3.25*	56.3
	24 h	10	37.59 ± 1.43*	42.9
	7 days	11	43.01 + 1.37**	34.6
Soman (90 μg/kg)	30 min	10	11.83 ± 1.59*	81.9
	24 h	10	19.78 ± 1.12*	69.9
	7 days	9	34.56 ± 0.59*	47.5

Values represent the mean ± S.E.M. Statistical analysis was performed with Kruskal–Wallis test followed by a post hoc pairwise Mann–Whitney test corrected by the Bonferroni procedure as described in Section 2 ($k = 45$ pairwise comparisons). Percent of inhibition was calculated using the mean values of cholinesterase activity.

* Statistical significance was set at $\alpha = .05$, $P < .0011$ (difference to control).

** Statistical significance was set at $\alpha = .01$, $P < .0002$ (difference to control).

with those obtained in the ChE activity study presented above. Mice were deeply anesthetized with pentobarbital and perfused transcardially with heparinized saline (5 IU/ml) followed by a fixative solution made of formaldehyde (4%) and acetic acid (3%). The entire brain was removed, postfixed for 6 h at 4°C and processed by routine paraffin embedding methods. Brain sections (6 μm) from bregma +1.1 to −2.18 mm (Franklin and Paxinos, 1997) were mounted onto slides precoated with 3-aminopropyl-triethoxy-silane. Neuropathology was evaluated by both the common hemalun–phloxin staining that effectively screens for neuronal necrosis with pycnotic nuclear changes, neuronal losses and neuronal edema (cells that undergo starting damage have a cytoplasmic acidophilia) and the in situ terminal deoxynucleotidyl transferase dUTP nick-end labeling method (TUNEL) (the TUNEL method allows the detection of free 3′-OH ends of DNA fragments that appear to be early evidences of apoptotic but also necrotic cell death, Graslkraupp et al., 1995; TUNEL kit: VQIA33, Amersham) according to the manufacturer's instructions.

2.4. Behavioral study

With respect to anxiety, the behavioral effects of soman were studied on an elevated plus-maze validated by Pellow et al. (1985) for rats and adapted by Lister (1987) for mice. The elevated plus-maze was made of Plexiglass and consisted of two open arms, 27 × 5 cm, and two enclosed arms, 27 × 5 × 15 cm, with an open roof arranged in such a way that the two arms of each type were opposite to each other. The plus-maze was elevated to a height of 40 cm. Randomly selected mice were injected with 30, 50 or 90 μg/kg of soman (or saline for controls) 30 min, 1 h or 7 days before the behavioral test. The different groups and the number of animals per group are listed in Tables 3, 4a and 5. Experimenters, unaware of the treatment group, randomly selected a mouse and placed it at the center of the plus-maze, facing an intersection between an open arm and an enclosed arm. During a 5-min test period, its position was recorded and analyzed by a CCD camera connected to a computer-assisted image processor (video-track, View-Point, Lyon, France). At the end of each test, the mouse was removed from the plus-maze, returned to its cage and the apparatus was cleaned with a paper towel. The following parameters were recorded: number of entries in the two open arms, number of entries in the two enclosed arms, time spent in the two open arms, time spent in the two enclosed arms, time spent in the center of the plus-maze, distance traveled in the plus-maze and in the five areas (central area and four arms). The following ratios are then calculated: number of entries in open arms/number of entries in the four arms, time spent in the open arms/time spent in the plus-maze, time

Table 3
Effects of soman (30 μg/kg) on performances in the elevated plus-maze 30 min, 24 h and 7 days after injection

Soman (30 μg/kg)	Number of animals	% distance in open arms	% time in open arms	% distance in enclosed arms	% time in enclosed arms	% open arms entries	Total distance (cm)
Control (30 min)	39	37.1 ± 2.0	38.8 ± 2.1	45.5 ± 1.8	36.0 ± 1.8	40.6 ± 2.4	1262 ± 25
Soman (30 min)	37	41.8 ± 1.6	45.6 ± 2.0*	42.1 ± 1.1	33.2 ± 1.6	44.4 ± 1.7	1191 ± 27
Control (24 h)	39	29.8 ± 1.5	32.1 ± 1.5	53.1 ± 1.6	42.8 ± 1.7	30.6 ± 2.1	1194 ± 24
Soman (24 h)	37	33.5 ± 1.9	35.8 ± 1.9	49.5 ± 1.5	39.5 ± 1.4	33.1 ± 2.3	1189 ± 29
Control (7 days)	39	33.5 ± 1.6	36.2 ± 1.8	48.5 ± 1.3	37.1 ± 1.3	34.3 ± 2.0	1277 ± 29
Soman (7 days)	37	33.9 ± 2.0	38.0 ± 2.0	48.1 ± 1.9	36.5 ± 1.8	34.1 ± 2.4	1232 ± 31

Values represent mean ± S.E.M. Control and soman groups were compared with Mann–Whitney test.

* $P < .05$, significantly different from the control group.

Table 4

(a) Effects of soman (50 μg/kg) on performances in the elevated plus-maze 30 min, 24 h and 7 days after injection

Soman (50 μg/kg)	Number of animals	% distance in open arms	% time in open arms	% distance in enclosed arms	% time in enclosed arms	% open arms entries	Total distance (cm)
Control (30 min)	39	33.6 ± 1.8	36.8 ± 2.0	46.0 ± 1.4	36.3 ± 1.5	35.5 ± 2.3	1399 ± 38
Soman (30 min)	37	27.5 ± 2.3*	28.8 ± 2.8*	53.3 ± 2.5*	44.2 ± 3.2	26.2 ± 2.7*	1013 ± 47***
Control (24 h)	39	26.5 ± 1.8	28.2 ± 1.8	53.0 ± 1.6	42.3 ± 1.8	27.5 ± 2.3	1223 ± 24
Soman (24 h)	37	30.0 ± 1.8	31.9 ± 1.9	49.8 ± 1.6	37.9 ± 1.6	30.7 ± 2.1	1171 ± 26
Control (7 days)	29	32.6 ± 2.4	35.4 ± 2.6	47.8 ± 2.1	37.6 ± 2.1	33.8 ± 2.8	1331 ± 36
Soman (7 days)	27	31.4 ± 1.9	35.1 ± 2.3	48.9 ± 1.6	36.8 ± 1.8	32.2 ± 2.6	1289 ± 36

(b) Spearman's rank correlation coefficients between total distance traveled in the elevated plus-maze and the other parameters of the test, 30 min after injection of soman (50 μg/kg) or its excipient

		Spearman's rank correlation coefficients *R* between total distance traveled vs.				
Treatment	Number of animals	% distance in open arms	% time in open arms	% distance in enclosed arms	% time in enclosed arms	% open arms entries
Control (30 min)	39	0.383* (*P* = .016)	0.414** (*P* = .009)	− 0.214 (*P* = .19)	− 0.196 (*P* = .23)	0.233 (*P* = .15)
Soman (50 μg/kg, 30 min)	37	0.462** (*P* = .004)	0.473** (*P* = .003)	− 0.343* (*P* = .04)	− 0.313 (*P* = .06)	0.269 (*P* = .11)

Values represent mean ± S.E.M. Control and soman groups were compared with Mann–Whitney test.

* $P < .05$, significantly different from the control group.

** $P < .01$, significantly different from the control group.

*** $P < .001$, significantly different from the control group.

spent in the enclosed arms/time spent in the plus-maze, distance traveled in the open arms/distance traveled in the plus-maze, distance traveled in the enclosed arms/distance traveled in the plus-maze. These ratios were then appropriately expressed respectively as percentage of the open arm entries, percentage of time spent in the open arms, percentage of time spent in the enclosed arms, percentage of time spent in the enclosed arms, percentage of distance traveled in the open arms, percentage of distance traveled in the enclosed arms. A compound is considered as possessing an anxiogenic effect when the percentage of entries, time spent or distance traveled in the open arms diminishes or when the percentage of entries, time spent or distance traveled in the enclosed arms increases. An opposite pattern leads to consider a compound as possessing an anxiolytic effect. The total distance traveled is an index of the locomotor and/or exploratory behavior. A decrease of distance may be due to a decrease of the exploratory behavior or to a locomotor impairment. In a previous pilot study (data not shown), the presently adopted test process was validated by using β-carboline-3-carboxylic acid methyl ester (βCCM) (Sigma, France) and diazepam (Hauffman La Roche, Bâle, Suisse), respectively, an anxiogenic and an anxiolytic compound. Both drugs have previously been widely tested in mice with the elevated plus-maze (e.g., Belzung and Agmo, 1997; Belzung et al., 1991; Johnson and Rodgers, 1996; Rochford et al., 1997).

Behavioral, biochemical and histological data were evaluated by different experimenters who were blind to the treatment conditions of the animals.

2.5. Statistics

Nonparametric statistics were used throughout the study with the Statistica 5.1 software (StatSoft France, 1997). Significance was set at 5%. Blood ChE data were analyzed by the Wilcoxon's test for dependent samples. Brain ChE values were compared using the Kruskal–Wallis test followed, when necessary, by post hoc pairwise comparisons using the Mann–Whitney test corrected by the Bonferroni

Table 5
Effects of soman (90 μg/kg) on performances in the elevated plus-maze 30 min, 24 h and 7 days after injection

Soman (90 μg/kg)	Number of animals	% distance in open arms	% time in open arms	% distance in enclosed arms	% time in enclosed arms	% open arms entries	Total distance (cm)
Control (30 min)	9	29.6 ± 4.7	31.8 ± 4.7	52.1 ± 3.1	41.2 ± 3.0	33.8 ± 6.0	1278 ± 71
Soman (30 min)	6	26.9 ± 9.6	16.7 ± 10.3	51.3 ± 13.3	53.2 ± 14.8	37.8 ± 16.4	548 ± 95***
Control (24 h)	9	27.5 ± 2.9	28.4 ± 3.1	55.2 ± 3.1	46.4 ± 3.0	32.0 ± 4.0	1315 ± 30
Soman (24 h)	7	32.1 ± 5.0	31.5 ± 7.1	53.5 ± 5.1	49.5 ± 8.6	36.7 ± 5.4	1018 ± 95*
Control (7 days)	9	33.0 ± 3.1	36.2 ± 3.2	50.8 ± 2.8	39.4 ± 2.6	36.5 ± 3.9	1345 ± 83
Soman (7 days)	7	29.9 ± 3.9	30.9 ± 4.5	53.2 ± 4.0	43.9 ± 3.9	32.3 ± 5.4	1118 ± 43

Values represent mean ± S.E.M. Control and soman groups were compared with Mann–Whitney test.

* $P < .05$, significantly different from the control group.

*** $P < .001$, significantly different from the control group.

procedure. Performances in the elevated plus-maze for soman-intoxicated mice were compared to those obtained with matched control mice using Mann–Whitney test. When indicated, the correlation between the total distance traveled and other behavioral parameters was assessed using Spearman's rank correlation test.

3. Results

3.1. Intoxication by 30 μg/kg of soman

3.1.1. Clinical observations

For this dose, no signs of poisoning were observed.

3.1.2. Anxiety behavior

Compared to the control group, only a significant increase of the percentage of time spent in the open arms was observed (Table 3).

3.1.3. ChE activity study

As shown in Tables 1 and 2, peripheral and central ChE activities were significantly reduced by 30 min after the intoxication (inhibition levels of $\approx 44\%$ and $\approx 35\%$, respectively). Afterwards, the ChE activity increased faster in the blood than in the brain. Nevertheless, significant inhibition remained observable in blood and brain 24 h after poisoning. Seven days after poisoning, central and whole blood ChE remained significantly inhibited. However, blood ChE activity was close to baseline value.

3.1.4. Histopathology

Histopathology did not differ from the controls ($n = 10$). Indeed, neither cell exhibiting pathological changes (hemalun–phloxin staining) nor DNA fragmentation were detected at 24 h ($n = 10$) or 7 days ($n = 10$) after intoxication (data not shown).

3.2. Intoxication by 50 μg/kg of soman

3.2.1. Clinical observations

The animals were hypoactive and showed occasional very weak tremors. Hypersalivation, marked tremors and convulsions were never observed.

3.2.2. Anxiety behavior

Compared to the controls, as shown by the total distance traveled, a slight hypolocomotion could be observed in most animals 30 min after poisoning (Table 4a). At the same time point, soman-intoxicated mice traveled longer distance in the enclosed arms than the controls. Conversely, they spent less time, traveled shorter distance in the open arms and entered less in the open arms (Table 4a). All of these suggest, in a first approach, increased anxiety. In a previous study (Lister, 1987), using picrotoxin, which possesses both anxiogenic and hypolocomotion effects, indicates that "an anxiogenic interpretation of picrotoxin's action should be made with caution since all the behavioral measures were depressed." Moreover, it is known that total arm entries is correlated with locomotion (Pellow et al., 1985; Lister, 1987). Globally, the possibility that the difference of percentage of open or closed arms entries could be the consequence of the hypolocomotion after soman poisoning could not be discarded. Thus, in order to evaluate if a link exists between the abovementioned hypolocomotion induced by soman and the increased anxiety observed, correlation tests were performed between the total traveled distance and the parameters of anxiety in animals of both control and 50-μg/kg soman groups. The results (Table 4b) clearly show that significant positive correlations exist, in both groups, between the total distance traveled and either the percentage of distance in open arms or the percentage of time in these arms. Moreover, in the soman group alone, the total distance traveled and the percentage of distance in the enclosed arms appear significantly and negatively correlated. Accordingly, the significant change observed 30 min after intoxication by soman, in the percentages of distance and time in open arms and the percentage of distance in enclosed arms, are likely related to the hypolocomotion. Conversely, the significant decrease of the percentage of open arms entries does not appear correlated with hypolocomotion, thus, reflecting a pure anxiogenic effect.

At 24 h and 7 days after intoxication, there was no difference on anxiety parameters between controls and soman-treated mice (Table 4a).

3.2.3. ChE activity study

Inhibition of central ChE (Table 2) was of 56.3% 30 min after intoxication, while blood ChE inhibition reached 63.3% (Table 1). Twenty-four hours after intoxication, ChE inhibition was still marked in both brain and blood ($\approx 43\%$ and $\approx 33\%$, respectively). As for the 30-μg/kg dose, 7 days after poisoning, central ChE remained significantly inhibited, while blood ChE activity returned to baseline value.

3.2.4. Histopathology

Histopathology did not differ from the controls ($n = 10$). Neither eosinophilic cell nor DNA breaking was detectable 24 h ($n = 12$) or 7 days ($n = 10$) after the injection of 50 μg/kg of soman (data not shown).

3.3. Intoxication by 90 μg/kg of soman

3.3.1. Clinical observations

For this dose, all of the animals presented typical signs of soman intoxication. Nevertheless, the clinical manifestations, except chewing and hypersalivation that could be observed in every animal, were different and variable in intensity. Indeed, the mice could show either slight tremors, marked tremors, marked tremors with convulsions of short duration (a few minutes) or marked tremors with long-

lasting convulsions (>1 h). This variability of clinical observations was in accordance with the expected responses for this threshold dose for convulsions.

3.3.2. Anxiety behavior

Thirty minutes after the injection of soman, one animal was excluded from the analysis because of frequent falls from the elevated plus-maze. Compared to the controls, a significant decrease of the total distance traveled in the elevated plus-maze, without any modification of the other tested parameters, was observed 30 min and 24 h after the intoxication (Table 5). These results reflect a strong hypolocomotion. Seven days after treatment, the performances were the same as for the controls.

3.3.3. ChE activity study

Thirty minutes after the intoxication, a similar high level ($\approx 80-85\%$) of inhibition was observed in brain and blood (Tables 1 and 2). Afterwards, as it was observed for the other lower doses of soman, the recovery of ChE activity occurred more quickly in blood than in brain. At the 7-day time point, ChE inhibition was still elevated in the brain ($\approx 48\%$), while blood level was slightly above the baseline value.

3.3.4. Histopathology

Among the 15 mice treated by 90 μg/kg of soman and observed 24 h after intoxication, only one, which had experienced convulsions during more than 4 h, showed numerous eosinophilic cells and TUNEL-positive cells (DNA fragmentation) in the lateral septum, the endopiriform and entorhinal cortices, the dorsal thalamus, the hippocampus and the amygdala (Fig. 1). At this same time point, in two other animals, which experienced obvious tremors with convulsions of short duration (during the prehension of the animal and the blood sampling), only some eosinophilic cells were seen in the thalamus ($n=1$) and in the cortex ($n=1$). No brain damage was detected in the other animals, which experienced only slight tremors but no convulsion. In the group that was observed for 7 days after intoxication ($n=19$), all the animals experienced only slight tremors and no convulsion. No histopathology was ever detected in any of these 19 animals.

4. Discussion

The early modifications in anxiety seen in our study after the injection of soman (30 and 50 μg/kg) may be linked to

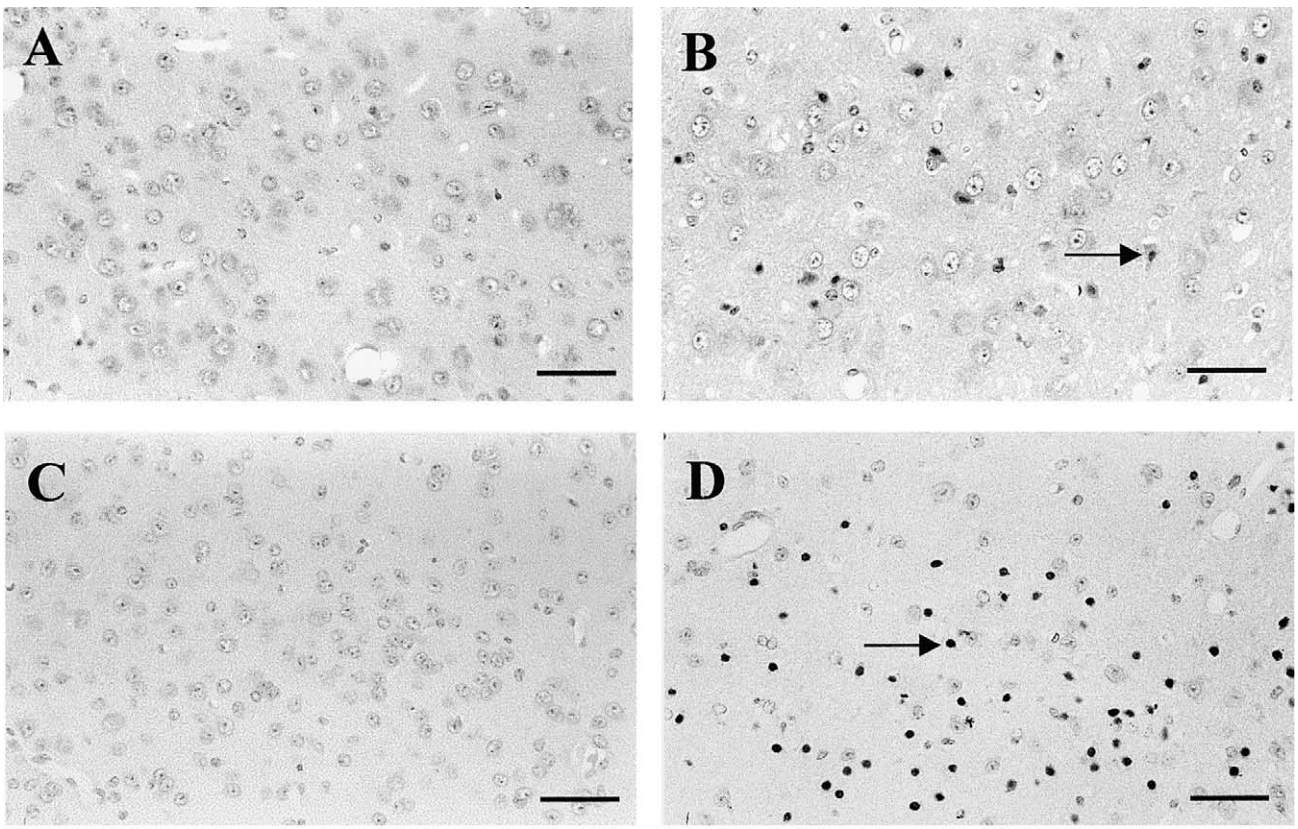

Fig. 1. Histopathology in the amygdala 24 h after intoxication by 90 μg/kg of soman. Hemalun–phloxin staining (A, B). DNA fragmentation (TUNEL reaction) (C, D). Note the absence of acidophilic cells and DNA fragmentation for the control (A and C, respectively), while numerous eosinophilic cells (B) and TUNEL-positive cells (D) (arrows) are detectable in one animal that experienced convulsions. Scale bar = 50 μm.

the soman-induced accumulation of acetylcholine (ACh) at central nervous system synaptic terminals. Indeed, the cholinergic system is known to play a modulator role in the control of anxiety (File et al., 1998; Ouagazzal et al., 1999a), this control being complex and depending on the state and/or type of anxiety (Ouagazzal et al., 1999b). An increase of ACh was previously observed after intoxication with one LD_{50} of soman (Lallement et al., 1992), but remains to be studied with lower doses.

For the 30-μg/kg dose of soman (0.27 LD_{50}), a slight and transient reduction of anxiety without histopathological changes was detected only 30 min after the intoxication with this nonconvulsant and asymptomatic dose of nerve agent. Comparatively, Sirkka et al. (1990) reported that a dose of 0.11 LD_{50} of soman led to an anxiogenic effect in rats. The discrepancies between the present study and that of Sirkka et al. may stem from differences in animal species and/or administration route of the toxic (ip vs. sc) and/or the latency between intoxication and the behavioral test. Indeed, as mentioned above, the cholinergic modulation of anxiety is known to be complex. In this view, the stimulation of nicotinic receptors may induce either anxiolytic or anxiogenic responses depending upon the duration of this stimulation (Olausson et al., 1999; Ouagazzal et al., 1999a). Whatever it is, since the level of blood and brain ChE inhibitions were not studied by Sirkka et al., no further precise comparison between their study and our study can be made.

Considering the symptomatic and subconvulsant 50-μg/kg dose of soman 30 min after poisoning, an increase of anxiety was noted as revealed by the decrease of percentage of open arms entries. Indeed, (a) for the other anxiety parameters (percentage of distance and time in open arms and percentage of distance in enclosed arms), correlation studies show that the significant differences observed are related to the soman-induced hypolocomotion and (b) as stressed by Lister (1987), a reduction of the percentage of entries (like a reduction of the percentage of time spent in the open arms) is consistent with an anxiogenic effect.

The reasons why 30 μg/kg of soman induced an early anxiolytic effect while, conversely, a dose of 50 μg/kg led to an anxiogenic effect are still unknown. However, it could be suggested that this biphasic response of soman might be explained by initial facilitation of synaptic transmission by slight ChE inhibition or enhancement of ACh availability in the synapse, which at overstimulation may turn to loss of function as known from, e.g., neuromuscular transmission.

On the other hand, as for convulsant doses of soman (Fosbraey et al., 1990; Lallement et al., 1991; Shih and McDonough, 1997), it is possible that, besides ACh, other neurotransmitter systems could also be disturbed after the administration of a subconvulsant dose of the compound. In this view, the involvement of the GABAergic system in anxiety requires careful consideration. The anxiolytic effects of benzodiazepines are well known and a correlation between anxiety and a decreased number of GABA and benzodiazepine receptors was demonstrated (Rago et al., 1988). In this context, it is noteworthy that previous studies demonstrated an early and transient decrease of $GABA_A$ergic function in the hippocampus (Lallement et al., 1993) within the first 30 min following a single dose of soman at one LD_{50}. Such an impairment of $GABA_A$ergic function may also be implicated only in the present anxiogenic effect observed early after the administration of the mild 50-μg/kg dose of soman, but not in the anxiolytic effect induced by the low 30-μg/kg dose of soman. Investigations on the consequences of these low to mild doses of soman on various neurotransmitter systems and especially the $GABA_A$ergic one would thus be of particular interest.

Finally, it must be noted that, in the animals treated with either 30 or 50 μg/kg of soman, the observed various behavioral impairments (hypolocomotion, anxiogenesis and anxiolysis) are not accompanied with any histopathological sequelae. Therefore, the behavioral changes certainly do not derive from gross cerebral morphopathological degradations.

Regarding the 90-μg/kg dose of soman, obvious signs of toxicity (marked tremors sometimes associated with convulsions) were observed. Moreover, an important decrease (59%) of the total distance traveled in the elevated plus-maze was noted 30 min after the intoxication indicating a dramatic hypolocomotion that approached a state of actual incapacitation. Such an effect was still detectable, although reduced, after 24 h but was no longer significant 7 days after soman administration. Consequently, neither anxiogenic nor anxiolytic responses to the intoxication could be evaluated 30 min and 24 h after administration of 90 μg/kg of soman. No modification in anxiety could be noted at the 7-day time point. Finally, neuropathology was shown to occur only in mice that experienced convulsions, thus, reaffirming the close relationship, which is known to exist between soman-induced neuropathology and seizure activity (Carpentier et al., 2000).

4.1. Additional comments on ChE inhibition by soman

It is known that the relative quantitative relationships between peripheral and brain AChE inhibition varies depending upon the specific OP (Storm et al., 2000). In the case of pesticide, it has been reported that ChE activity in whole blood correlated well with ChE activity in the brain (Padilla et al., 1994). To our knowledge, no such data are available for nerve agents. All doses together, the present results show that ChE activity recovered relatively more quickly in whole blood than in brain. Indeed, blood ChE inhibition was consistently lower than the one observed in brain. Although blood ChE activity was apparently still depressed 7 days after intoxication, the percentage of inhibition may lie within the known variability of ChE activity values as stressed by the conflicting results obtained with 30 and 50 μg/kg. Thus, beyond the early first hours after soman intoxication, blood ChE inhibition level did not appear to be correlated with the inhibition in the

brain, the discrepancy being more pronounced as the dose of soman was increased. Present study regards the whole blood ChE activity reflecting both erythrocytes acetylcholinesterase (RBC AChE) and plasma ChE including notably butyrylcholinesterase (BuChE). The recovery after inhibition is known to differ between AChE and BuChE (e.g., Gupta et al., 1987b; Lim et al., 1989). The rapid recovery of blood ChE in the present study may be linked to known faster plasma BuChE regeneration by synthesis of protein in the liver (e.g., Gupta et al., 1987a) compared to the recovery of RBC AChE activity, which only depends on the regeneration of erythrocytes (erythrocytes do not have the ability of protein synthesis). According to our observations, Grubic et al. (1981) and Yaksh et al. (1975) demonstrated that, after intoxication by soman, plasma ChE recovered faster than brain AChE. Consequently, the use of blood ChE level as the sole biological index of the severity of soman intoxication (Pope and Chakraborti, 1992; Ray, 1998; Yokoyama et al., 1998) must be considered cautiously since, depending on the latency between intoxication and blood sampling, it may not be accurately correlated with the recovery of enzymatic activity in the central nervous system.

In conclusion, the combined study of the level of brain ChE inhibition and of the behavioral and histopathological consequences of soman intoxication shows that, in the central nervous system, (a) inhibition of $<35\%$ (measured 30 min postintoxication) produces an early and transient anxiety behavioral alteration but no histopathological sequelae, (b) ChE inhibition of 50–60% leads both to an early and transient anxiogenic effect and to hypolocomotion without histopathological injury and (c) an inhibition of 80% induces severe incapacitation, whereas brain damage is found only in animals experiencing major tremors or convulsions. All in all, this study demonstrates that, for low doses of nerve agent anxiety, changes or locomotor disturbances could be observed even in the absence of any neuropathology. Nevertheless, as the present data only concerned a period of time up to 7 days, further studies should look beyond this time frame to assess later changes in pathology or behavior after soman intoxication with low to mild doses.

From an applied point of view, these effects of an acute low dose of OP administration, subtle and transient as they are, may be considered in a first approach as a nondetrimental epiphenomena. However, they must be taken into serious consideration since the stressful conditions of the battlefield or the panic that follows a terrorist attack with a nerve agent might dangerously influence the impairments caused by a mild dose of the compound itself.

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