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Scopolamine Augments the Efficacy of Physostigmine Against Soman Poisoning in Guinea Pigs

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PHILIPPENS, I. H. C. H. M., B. P. C. MELCHERS, B. OLIVIER AND P. L. B. BRUIJNZEEL. *Scopolamine augments the efficacy of physostigmine against soman poisoning in guinea pigs.* PHARMACOL BIOCHEM BEHAV **65**(1) 175–182, 2000.—The efficacy of the subchronically administered cholinesterase-inhibitor physostigmine (PHY) (0.025 mg/kg/h) either with or without the muscarinergic receptor antagonist scopolamine (SCO) (0.018 mg/kg/h) in counteracting soman-induced lethality and incapacitation were determined in guinea pigs. This was tested in animals that either received atropine sulphate (AS, 17.4 mg/kg IM) or no postintoxication therapy. Behavioral and neurophysiological readout systems were used to measure postintoxication incapacitation. Only the pretreatment with PHY alone did not offer any protection against 2× LD_{50} soman intoxication. Animals that received the complete treatment (PHY + SCO + AS) did not show any abberations in the performance of learned behavior. The use of AS after soman intoxication resulted in an increase of the startle response, whereas the addition of SCO to the pretreatment led to a more persistent duration of the effect in time. In case one has to rely completely on the pretreatment, the addition of SCO to PHY is life-saving. However, some postintoxication incapacitation is still present. Therefore, the pretreatment regime may perhaps further be improved by the addition of a nicotinic antagonist. © 1999 Elsevier Science Inc.

Behavior Cholinesterase Guinea pig Physostigmine Pretreatment Scopolamine Soman Subchronic

THE toxicity of organophosphorus (OP) compounds is known to be due to their acetylcholinesterase (AChE) inhibition in the synaptic cleft (31). Protection against this type of intoxication can be achieved by pretreatment with a carbamate that inhibits the enzyme AChE in a reversible manner (18). Their prophylactic activity in reducing the toxic effects of the OP soman has already been shown (4,18). Currently, the carbamate pyridostigmine (PYR) containing a quaternary nitrogen is used as a pretreatment against OP intoxication. However, due to the quaternary nitrogen, PYR can hardly pass the blood–brain barrier. Therefore, PYR only protects peripheral AChE. On the other hand, OPs easily penetrate into the brain because of their highly lipophilic nature, thereby causing both peripheral and central toxicity. For this reason a pretreatment against OP intoxication should not only protect the peripheral compartment but also the brain. Therefore, physostigmine (PHY) has been proposed as an alternative for PYR (17). Leadbeater et al. reported PHY to be very effective against sarin or soman intoxication. The centrally active carbamate PHY (containing a tertiary nitrogen) protects more effectively against soman intoxication than PYR (13,28). Rats pretreated with PHY recovered completely from soman intoxication within hours, whereas in rats pretreated with PYR, symptoms lasted for 1 week (13). Recently, we have been able to confirm these data in guinea pigs (25). Guinea pigs pretreated with PHY appeared to be protected much better against soman-induced lethality than those pretreated with PYR. However, a pretreatment not only

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should be protective, it also should be devoid of side effects. Most of the side effects of the PHY pretreatment are due to AChE inhibition in the central nerve system (CNS). These effects can be counteracted by the muscarinic receptor antagonist scopolamine (SCO) (17). Indeed, the addition of SCO to PHY antagonized the side effects in a memory task and in the EEG after single-dose administration of PHY to guinea pigs (23). When PHY was applied subchronically, no side effects were observed in both guinea pigs and marmoset monkeys (25,26). When PHY is applied subchronically (via an osmotic mini pump) SCO may not be necessary as a supplement to PHY therapy. However, the addition of an antimuscarinic drug may improve the efficacy of PHY pretreatment (8,12,17). The question addressed in this study, therefore, was: Is additional therapy with SCO necessary to improve the protective efficacy of the pretreatment against intoxication with $2\times$ LD_{50} soman or does an adequate pretreatment regime offer sufficient protection by itself? Also, we addressed the question whether the addition of SCO reduced the postintoxication incapacitation effects. These questions are relevant because in case of nerve gas exposure in a war situation soldiers have to inject themselves with a postintoxication therapy. One can imagine situations that soldiers are deprived of or are not capable of using this type of therapy. In that situation, they have to rely completely on the pretreatment received. Therefore, the efficacy of the pretreatment was tested in a situation in which the animals did receive a postintoxication therapy with atropine sulphate, and in a situation in which the animals did not. Behavioral and neurophysiological test methods were used to determine the soman-induced postintoxication incapacitation in atropinezed or not atropinezed guinea pigs pretreated with PHY including or excluding SCO.

METHOD

Animals

Male Dunkin–Hartley albino guinea pigs CrL:(HA)BR (Charles River) with an initial body weight of 400–450 g were used. The animals were kept singly in a cage (Makrolon type IV). The ambient temperature was regulated between 20–22°C. Relative humidity was monitored but not regulated, and was kept over 50%. Food and water were always available. The here described experiments received prior approval by an independent animal ethical committee.

Drug Solutions and Implantation of Osmotic Minipumps

Physostigmine (eserine) and scopolamine bromide were obtained from Sigma (St. Louis, MO), atropine sulphate was obtained from ACF (Amsterdam, The Netherlands), soman (*O*-pinacolyl methylphosphonofluoridate) was synthesized at the Prins Maurits Laboratory TNO (Dr. H. P. Benschop).

Alzet® Osmotic Minipumps, with a constant delivery rate of 0.5 μl/h (Model 2002, Alza Corp., Palo Alto, Ca), were used to deliver either the vehicle, PHY (0.025 mg/kg/h) or the combination of PHY (0.025 mg/kg/h) and SCO (0.018 mg/kg/h). This dose of PHY offers the recommended blood–AChE inhibition of about 35% (25). SCO (0.018 mg/kg/h for a period of 10 days) leads to a SCO plasma concentration of 45 nM (25). This was comparable with the level found after a single SC injection of 0.1 mg/kg SCO (43 nM). This SCO plasma concentration did not lead to side effects on shuttlebox performance and on the startle reflex, and antagonized some of the PHY-induced side effects (21,23). The vehicle consisted of 20% propylene glycol, 10% ethanol, and 70% water (one part glacial acetic acid in 2000 parts distilled water). The drugs used were dissolved in the vehicle. Because the animals gain weight during the 2 weeks of the pretreatment period, the PHY and SCO concentrations were based on the estimated weight of the animals 1 week after implantation. This estimation was based on the normal growth curve for guinea pigs in our laboratory. The pumps were implanted subcutaneously under the skin on the backs of the animals under halothane/N_2O anesthesia. The wounds were sutured with woundclips.

Study Design

The here-described study was performed in four different treatment groups of animals ($n = 6$ or 7 animals/group). Two groups were pretreated with PHY (0.025 mg/kg/h) subchronically and two groups with the combination of PHY (0.025 mg/kg/h) and SCO (0.018 mg/kg/h). One group of each set of the different pretreatment groups received a postintoxication therapy with atropine (AS) (17.4 mg/kg IM) 1 min after a dose of $2\times$ LD_{50} soman intoxication (day 11) (PHY/AS and PHY/SCO/AS groups). The remaining two groups did not receive any therapy after soman intoxication (PHY and PHY/SCO groups). The LD_{50} dose of soman (subcutaneously) used was 24.5 μg/kg (10). The protection of the different treatment regimes against lethality and postintoxication incapacitation after soman poisoning was tested. Furthermore, the pretreatment with PHY or PHY/SCO was checked for the occurrence of side effects on behavior and neurophysiology (see below).

After the animals were trained in the shuttlebox, electrodes for the measurement of EEG and visual evoked response (VER) were fitted. To obtain control values, the body weight, blood–AChE activity, shuttlebox, startle response, EEG, and VER were registered/determined before implantation of the Alzet osmotic minipumps. Subsequently, based on the obtained results, four matched subgroups of six animals each ($n = 7$ in the PHY/AS group) were formed that showed no significant differences in any of the behavioral tests. Thereafter, Alzet osmotic pumps, containing either vehicle with PHY or a combination of PHY/SCO, were implanted. This was called day 0.

Side Effects

To measure the AChE inhibition during the subchronic pretreatment with PHY or PHY/SCO blood samples were collected from the ear vein at 3, 7, and 11 days (day 3, 7, and 11) after osmotic minipump insertion. The effects on behavioral and neurophysiological parameters were tested during the first week of subchronic pretreatment (see Table 1).

Protection Against Lethality Induced by $2\times$ LD_{50} Soman

The efficacy of subchronic PHY or PHY/SCO pretreatment with or without AS therapy in counteracting soman-induced lethality was investigated 11 days after implantation of the osmotic minipumps. The osmotic pumps were not removed. For the determination of AChE inhibition, blood samples were collected from the ear vein 1 and 2 h after soman intoxication. The lethality was determined at 24 and 48 h after soman intoxication.

Protection Against Postintoxication Incapacitation After Intoxication by $2\times$ LD_{50} Soman

The efficacy of subchronic PHY or PHY/SCO pretreatment with or without AS therapy in counteracting soman-induced postintoxication incapacitation effects was investi-

TABLE 1
TEST PROTOCOL OF ALL TREATMENT GROUPS

Test Days	Blood AChE Activity	Body Weight	Shuttlebox	Startle Response	EEG and VER
3	X		X	X	
4		X			
5					X
7	X	X	X	X	
11	XXX	X	X	X	X
12		X	X	X	X
13		X	X	X	
14		X		X	X
18			X		

Day 0 = implantation of the osmotic pump; day 11 = intoxication with soman; "XXX" = measures of AChE before, 1 and 2 h after intoxication.

gated by observing the postintoxication symptoms like hypersalivation, tremors, and convulsions, and by measuring behavioral and neurophysiological parameters. The observation of the symptoms started immediately after soman intoxication by investigators unaware of the treatment. After the intoxication symptoms became less severe, the animals were able to perform in the behavioral and neurophysiological test systems. These tests started 2 h after soman intoxication (day 11), and were repeated at different days (see Table 1).

Behavioral Tests

Shuttlebox performance. In this test the active avoidance of an unpleasant event upon a conditioned stimulus (CS) is used to measure the retrieval of learned behavior. For this test, an automated two-way shuttlebox, consisting of two equal compartments of 23 × 23 × 23 cm with rounded corners, connected by a photocell-guarded gate, is used. The animals have to learn how to avoid a stream of air (about 6 l/s, air tube diameter 1 cm) aimed at their fur within 10 s after presentation of a sound stimulus (CS). During the daily training and test sessions the animals receive 20 trials at an intertrial interval of 20–30 s (random). Only animals that reach the criterion of 80% or more correct avoidance reactions (CARs) after training, were used in the experiments [for details see (21)]. The number of CARs was used to express the active avoidance performance.

Auditory startle response. In this test the stretching movement of the hindpaws is used to measure the reaction of the animal on a startle signal (7). For this test the animals are exposed to 20 auditory startle pulses (120 dB, 10 kHz, 20 ms) while standing with their hindpaws on a platform in a vertically mounted PVC-tube (diameter 7 cm, length 16.5 cm). The startle response of 200-ms duration is measured by a transducer connected with the platform, registering the force exerted by the animal upon presentation of the stimulus. An AD converter of an IBM-compatible PC digitized the responses. The area under the curve (AUC), amplitude, and latency of the startle response are registered, and used to express the motor reaction of the startle reflex.

Neurophysiological Measurements

Under halothane/N_2O anesthesia a silver electrode is placed into a small hole in the skull, 3 mm lateral to the sutura sagitalis and 8.5 mm caudal from the sutura frontoparietalis, leaving the dura mater intact. A reference electrode is placed over the nasal cavity. Both electrodes are connected with a plug, and fixed on the skull with dental cement. During the test, the animals are immobilized in a vertically mounted PVC tube (for the startle response), and a transmitter is connected to the plug for telemetric registration of the EEG and VER. All EEG signals were amplified (50,000×), filtered (between 0–30 Hz for EEG and 0–500 Hz for VER), and fed into an AD converter of an IBM-compatible PC; sampling frequency was 50 Hz for EEG and 1 kHz for VER.

EEG registrations. Fast Fourier transformation (FFT), to obtain power spectra, is performed from five randomly chosen EEG epochs of 12 s out of a total recording time of 5 min. The obtained power spectra of the guinea pigs are averaged per group and subdivided into eight frequency classes (delta1: 0.8–2, delta2: 2–3.5, theta1: 3.5–5.5, theta2: 5.5–7.5, alpha1: 7.5–10, alpha2: 10–12.5, beta1: 12.5–18, beta2: 18–25 Hz). The total power (V^2) of the different frequency classes are used for the evaluation of the brain activity.

Visual evoked response (VER) measurements. For registration of the VER, the animals receive 30 light stimuli with a time interval of 2 s ± 20%. Following the stimuli, the EEGs are registered during 250 ms, and the responses averaged. For evaluation of effects the latencies and amplitudes of the positive (P1, P2, P3, P4) and negative (N1, N2, N3) peaks are measured and compared with the baseline values.

Determination of AChE Activity

Blood samples (25 μl) obtained from the ear vein of the guinea pig were immediately mixed with 1% saponin (BDH, Poole, UK), frozen in liquid nitrogen, and stored at −70°C. After appropriate dilutions, AChE activity was assessed using a radiometric method (15). The ACh end-concentration used was 12 mM; [^{3}H]ACh iodide (NEN, Dreieich, Germany) was diluted to a specific activity of 602 MBq·mmol^{-1}. Electric eelAChE was used as a reference.

Statistics

For statistical analysis of the behavioral tests an analysis of variance (ANOVA) was used, and for the neurophysiological tests a paired *t*-test. For the survival and symptomatology after soman intoxication a Fisher exact-probability test was used. In all tests p-values < 0.05 were considered significant.

RESULTS

First, the occurrence of side effects after subchronically administered PHY ($n = 13$) or PHY/SCO ($n = 12$) was examined. Thereafter, two aspects were studied: protection against lethality, and against postintoxication incapacitation induced by 2× LD_{50} soman.

Side Effects of Subchronic Administration of PHY or PHY/SCO

Body weight. All the animals of both pretreatment regimes (PHY, $n = 13$ and PHY/SCO, $n = 12$) gained weight amounting 40–60 g during the pretreatment period of 11 days. This fits in the normal growth curve for guinea pigs in our laboratory.

Blood–AChE inhibition. The mean blood–AChE inhibition of the subchronic PHY-treated animals ($n = 13$), measured as a percentage of their control value before osmotic pump implantation was: 20.3 ± 2.0%; for the PHY/SCO-treated animals ($n = 11$; in one animal the blood sample was discarded) it was: 21.2 ± 5.2%.

Side effects on behavioral parameters. In the shuttlebox task, none of the two sessions showed any aberration on the performance (CAR) in both groups compared with their baseline value after training (PHY: 17.3 ± 0.63 and 1.64 ± 0.8 (baseline: 16.6 ± 0.56), PHY/SCO: 18.6 ± 0.5 and 18.4 ± 0.4 (baseline: 17.7 ± 0.7)). On the amplitudes and AUCs of the startle response, measured at days 3 and 7 after pump insertion, no aberrations were found compared with the baseline values. The amplitudes measured at day 7, expressed as a percentage of the baseline value, was in the PHY groups 119.7 ± 29, and in the PHY/SCO group it was 94.1 ± 18 (ANOVA: $p > 0.05$).

Side effects on neurophysiological parameters. EEG: the total band power (V^2) in the different frequency classes of both groups (PHY or PHY/SCO) showed no difference compared with their baseline values. VER: neither the amplitudes nor the latencies latency of each positive and negative peak changed in both groups (PHY or PHY/SCO) compared with their baseline values (*t*-test; $p > 0.05$).

Protection Against Lethality Induced by 2× LD_{50} Soman

Body weight. The body weight of the animals in the groups treated with AS after soman intoxication was not affected when measured 24 h after the intoxication. The animals in the PHY/SCO group showed significant loss in body weight [from 584 ± 17 g to 534 ± 11 g ($n = 5$); $p = 0043$]. The body weights of the animals from the PHY group could not be measured because they all deceased within 24 h after soman intoxication.

Blood–AChE inhibition. The mean blood–AChE inhibition measured 1 and 2 h after soman intoxication of the different treatment groups of animals compared to the control values before pump implantation are shown in Table 2. There are no significant differences in AChE inhibition in the different groups.

Protective efficacy against soman-induced lethality. All animals of the PHY/AS ($n = 7$) and the PHY/SCO/AS ($n = 6$) groups survived the intoxication with 2× LD_{50} soman completely, and only one animal from the PHY/SCO group ($n = 6$) died after 13 min. Of the PHY-treated group, all the animals died after soman intoxication. Five guinea pigs of this group deceased 1 h, and one guinea pig 4 h, after soman intoxication. Soman-induced lethality was significantly higher in the latter group compared with the other treatment groups (Fisher exact-probability test, $p < 0.05$, two tailed).

Protection Against Postintoxication Incapacitation after 2× LD_{50} Soman Intoxication

Postintoxication incapacitation symptoms. Subchronic PHY pretreatment and AS therapy against 2× LD_{50} soman: most animals in this group only showed mild tremors, some ataxia, and muscle fasciculations, lasting from 7 min after intoxication till about 1.5 h after intoxication. Three out of seven animals showed convulsive activity lasting for periods of about 4 min. No signs of dyspnoea were noticed. The day after soman intoxication all the animals suffered from hypersalivation. This was not found in the groups of animals that received SCO in their pretreatment regime.

Subchronic PHY/SCO pretreatment and AS therapy against 2× LD_{50} soman: the animals of this group appeared to be in a better condition than the animals in the other three groups. The appearance of signs started about 10 min after soman intoxication. This was significantly later compared with the other groups (Fisher exact-probability test, $p < 0.05$, two tailed). All animals showed mild to severe tremors. Only two animals suffered from convulsions (lasting 2 and 23 min). No signs of dyspnoea were noticed. The signs disappeared after 1.5 h, and most animals were in a healthy condition.

Subchronic PHY pretreatment against 2× LD_{50} soman: most animals (five out of six animals) showed severe tremors and convulsions lasting for periods of 7 to about 20 min. The other animal showed slight tremors and some ataxia followed by a period of dyspnoea. During the convulsive activity the animals started to suffer from dyspnoea, which lasted until they died. All the animals in this group also showed effects on their eyes. Their eyes enlarged and "edematous" hydrolated. The fist symptoms started within 5–10 min after intoxication. The clinical symptoms of the animals in this group were significantly worse compared with the groups treated with AS (Fisher exact-probability test, $p < 0.05$, two tailed).

Subchronic PHY/SCO pretreatment against 2× LD_{50} soman: the animals of this group were in a much worse condition compared to those treated with AS after soman intoxica-

TABLE 2

BLOOD AChE INHIBITION AFTER 2× LD_{50} SOMAN INTOXICATION IN THE DIFFERENT TREATED GROUPS OF ANIMALS

	AChE inhibition in % (±SEM)			
	PHY	PHY/SCO	PHY/SCO/AS	PHY/AS
1 h after soman	87.0 (0.9)	88.5 (1.0)	87.2 (1.4)	85.6 (0.6)
2 h after soman	90.9 (1.9)	91.2 (0.8)	89.0 (1.0)	89.6 (1.8)

Mean ± SEM, $n = 6$/group.

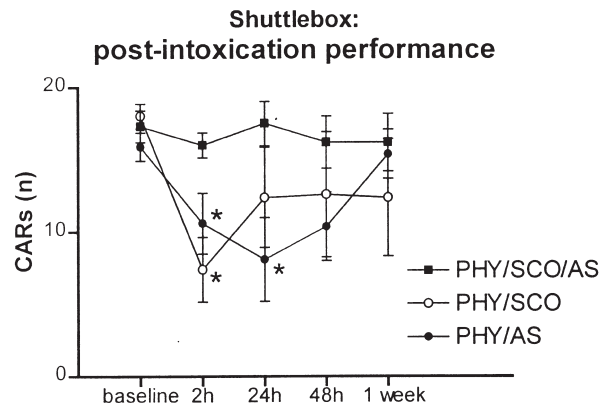

FIG. 1. Postintoxication effects on the shuttlebox performance after training and before pump insertion (baseline) and 2, 24, and 48 h and 1 week after soman (2× LD_{50} SC) intoxication (number correct avoidances, mean ± SE). Three groups were tested: PHY/SCO (n = 5), PHY/SCO/AS (n = 6), and PHY/AS (n = 7). The animals were pretreated with PHY (0.025 mg/kg/h) alone or combined with SCO (0.018 mg/kg/h). Two groups received a treatment with AS (17.4 mg/kg IM) 1 min after soman intoxication. *Significant compared with the baseline value ($p < 0.05$).

tion. Four out of six animals showed convulsions lasting for periods of 2, 3, 20, and 23 min, followed by a period of muscle fasciculations and dyspnoea. Only the animal that showed convulsions for 23 min did not suffer from dyspnoea, and the animal that suffered only 2 min from convulsive activity died very shortly after the soman intoxication. The remaining animals all suffered from severe tremors and dyspnoea. The signs started about 8 min after soman intoxication, and lasted until 2.5 h. The duration of the symptoms was significantly longer compared with the two groups that received AS therapy (Fisher exact-probability test, $p < 0.05$, two tailed).

Postintoxication incapacitation effects on behavioral measurements. The postintoxication incapacitation effects on the shuttlebox performance after soman intoxication are shown in Fig. 1. All the animals that survived the intoxication were able to perform the task in the shuttlebox; they showed a normal intertrial response (ITR) activity (compartment changes during the intertrial interval). The animals from the PHY/SCO/AS group performed very well in this task, whereas the animals from the PHY/AS, like those of the PHY/SCO group, showed a significant decrease of their CARs (an ANOVA analysis at 4 h after soman showed $p = 0.042$ and $p = 0.006$, respectively, and at 24 h after soman $p = 0.033$ for the PHY/AS group). One week later the performance of the animals in the PHY/AS group returned to normal baseline value. At that moment the performance of the PHY/SCO group was still affected (not significant). This was mainly due to the fact that the animals that performed very poorly in this task shortly after soman did not recover during that week, whereas the animals that only showed a mild decrease of the performance recovered almost completely after 1 week.

The effects on the startle response observed after 2× LD_{50} soman intoxication are shown in Fig. 2. In all the groups an increase of the startle response was observed. The results indicate that a single dose of AS enhanced the effect on the startle reflex after soman intoxication in PHY- or PHY/SCO-pretreated animals, whereas the addition of subchronically administered SCO increased the duration of the effect.

The animals from the PHY/AS group showed an increase of the startle response shortly after soman intoxication. This increase affected the AUC significantly ($p = 0.02$) at 2 h after soman intoxication. Furthermore, at this time point also the latency of the response was significantly delayed ($p = 0.013$). The animals from the PHY/SCO/AS group showed a significant increase of the startle response measured at 24 h after soman intoxication (amplitude: $p = 0.021$, AUC: $p = 0.025$). The effects on the startle response in the PHY/SCO group were not found to be significant.

Two days after soman intoxication the startle responses of all the groups were back to their baseline values. The animals of the PHY group could not be measured because they died before the start of the test. Only one animal of the PHY group that deceased 4 h after soman was tested in the startle response task. This animal showed a very weak response.

Postintoxication incapacitation effects on neurophysiological measurements. The EEGs were measured 2, 24, and 72 h after soman intoxication (data not shown). In the PHY/SCO/

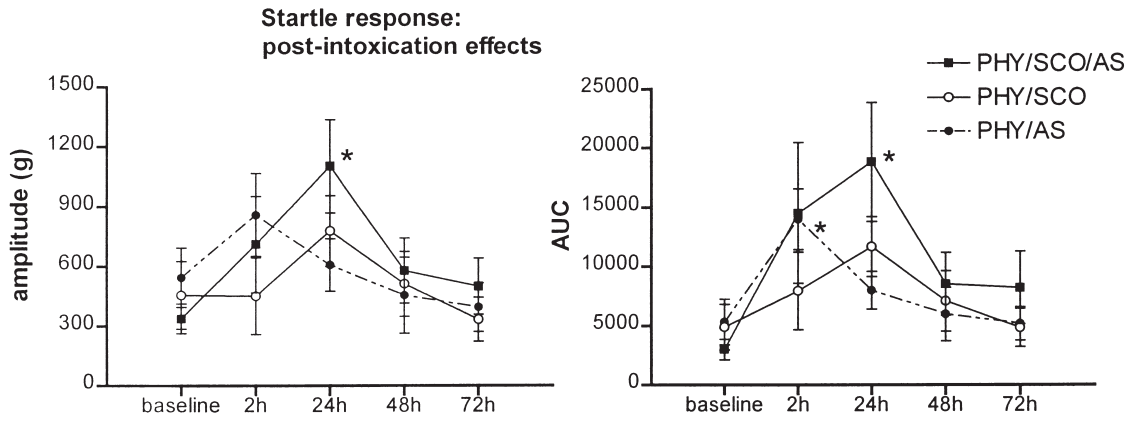

FIG. 2. Postintoxication effects on the startle response amplitude and AUC before pump insertion (baseline) and 2, 24, 48, and 72 h after soman (2× LD_{50} SC) intoxication (mean ± SE). Three groups were tested: PHY/SCO (n = 5), PHY/SCO/AS (n = 6), and PHY/AS (n = 7). The animals were pretreated with PHY (0.025 mg/kg/h) alone or combined with SCO (0.018 mg/kg/h). Two groups received a treatment with AS (17.4 mg/kg IM) 1 min after soman intoxication. *Significant compared with the baseline value ($p < 0.05$).

AS group, a significant decrease of the power was found in the alpha2 and beta1 bands 2 h after the intoxication ($p = 0.0001$ and $p = 0.024$). In the PHY/SCO group, a significant increase of the power was found only in the alpha2 band 24 h after the intoxication ($p = 0.0003$) and in PHY/AS group in the alpha1 band 72 h after the soman intoxication ($p = 0.027$). No effects were found i nthe delta and theta bands.

The VERs of the different treatment groups measured at 2, 24, and 72 h after soman are shown in Figs. 3 and 4. The peak amplitudes and latencies of each animal were measured, averaged per treatment group, and compared with their own control values. For all test points in the different groups no effect was found on the latency of the VER peaks after 2× LD_{50} soman intoxication (at all registration points $p > 0.05$). The amplitudes of the responses, on the other hand, seem to be affected after soman exposure. An increase of the N3 peak was found in all the groups at 2 h after the soman intoxication and in the AS-treated groups at all registration points. This was found to be worst in the PHY/AS group, but not to be significant because of the variation between the animals. Furthermore, a slight but not significant increase was found of the N2 peak, especially in the PHY/AS group (at all registration points $p > 0.05$).

DISCUSSION

In this study, the efficacy of the addition of SCO to the subchronic PHY pretreatment in a therapeutically relevant dose (9), against 2× LD_{50} soman was tested. This was done by comparing both pretreatment regimes in the absence or presence of a postintoxication therapy with AS. Two aspects were studied: the protection against lethality induced by 2× LD_{50} soman, and the protection against postintoxication incapacitation after intoxication by 2× LD_{50} soman.

Before the efficacy of the different treatment regimes was examined the pretreatment with PHY or PHY/SCO was tested for the occurrence of behavioral and neurophysiological side effects. In the tests used, no behavioral or neurophysiological side effects were found during subchronic pretreatment with PHY or the combination of PHY and SCO (18 μg/kg/h). These findings confirm the results found in an earlier study with guinea pigs (25). In that study, almost the same protocol was followed for testing side effects of PHY or PHY/SCO pretreatment.

Protection Against Lethality Induced by 2× LD_{50} Soman

The use of a postintoxication therapy with AS enhanced the protection against soman induced lethality. Animals that received AS immediately after soman intoxication survived completely. This was also found in an earlier study in which the efficacy of PHY or PHY + SCO pretreatment against soman intoxication was tested in the presence of a postintoxication therapy with AS (25). On the other hand, PHY-pretreated animals that did not receive a postintoxication therapy were not protected against the toxicant. They all deceased about 1 h after soman. It has been reported in the literature that guinea pigs only pretreated with acute PHY all died within 30 min of 2× LD_{50} soman administration (19). When a single dose of AS was given after soman intoxication, the protective ratio of a carbamate, like PYR or PHY, was considerable improved (11,17,20). This is noteworthy, because the protective ratio of AS alone has been reported to be 1.5 (14).

When the PHY pretreatment was combined with SCO, the animals were able to withstand the soman intoxication; only one animal died. It appeared that the blood–AChE of this an-

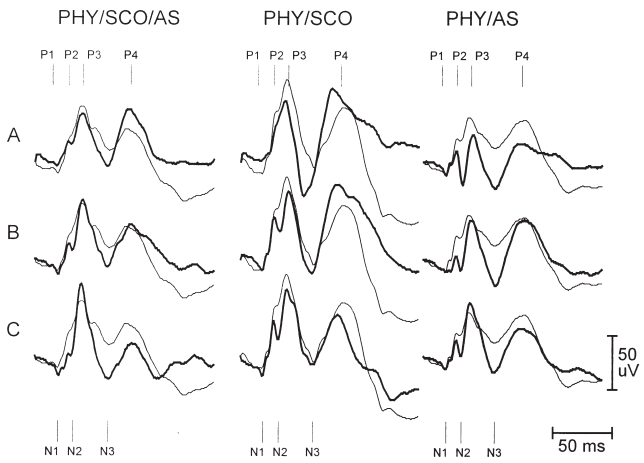

FIG. 3. Postintoxication effects on the VER (thick lines) compared with the baseline values (before pump insertion, thin lines) registered at (A) 2, (B) 24, and (C) 72 h after soman (2× LD_{50} SC) intoxication. Three groups were tested: PHY/SCO/AS ($n = 6$), PHY/SCO ($n = 5$), and PHY/AS ($n = 7$). All animals were pretreated with PHY (0.025 mg/kg/h) alone or combined with SCO (0.018 mg/kg/h). Two groups received a treatment with AS (17.4 mg/kg IM) 1 min after soman intoxication. The averaged VER curves from equally treated guinea pigs are shown in which the four positive peak (P1, P2, P3, P4) and three negative peaks (N1, N2, N3) are indicated.

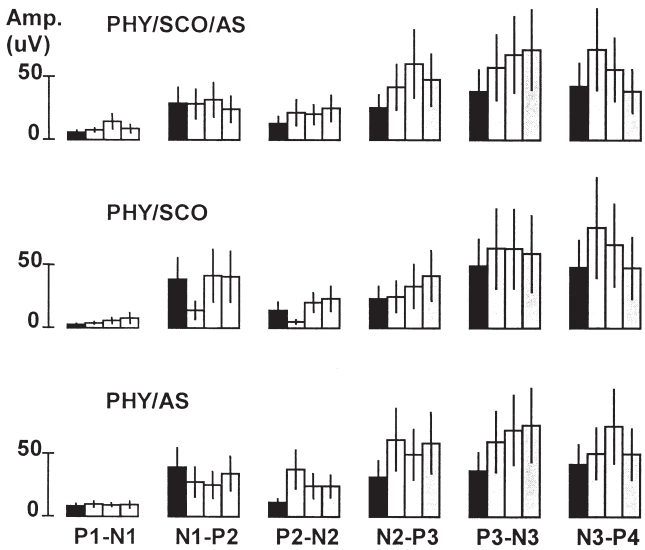

FIG. 4. Postintoxication effects on the VER amplitude expressed as the voltage difference between two sequential peaks (mean ± SE). Three groups were tested: PHY/SCO/AS ($n = 6$), PHY/SCO ($n = 5$), and PHY/AS ($n = 7$). The animals were pretreated with PHY (0.025 mg/kg/h) alone or combined with SCO (0.018 mg/kg/h). Two groups received a treatment with AS (17.4 mg/kg IM) 1 min after soman intoxication. Baseline values were measured and averaged per treatment group before pump insertion (black bars). Effects after soman (2× LD_{50} SC) intoxication were measured at 2 h (hatched bars), 24 h (open bars), and 72 h (double-hatched bars). No significant differences were found (at all registration points $p > 0.05$).

imal was not inhibited during the pretreatment period. Presumably, the osmotic pump had not delivered PHY + SCO properly, resulting in insufficient pretreatment. Indeed, the survival time after soman intoxication of this animal was as short (13 min) as in untreated animals. A higher protection against soman-induced lethality by addition of SCO to the PHY pretreatment has been reported in literature (17,19,20). Remarkably, SCO alone did not offer any protection against soman induced lethality (32). It can be concluded that addition of SCO to PHY as a pretreatment or addition of AS as postintoxication therapy enhance the protection synergistically against soman-induced lethality. In case where postintoxication therapy is not used, the addition of SCO in the pretreatment regime is of life-saving importance.

Protection Against Postintoxication Incapacitation After 2× LD_{50} Soman

The treatment regime consisting of a pretreatment with PHY and SCO followed by a postintoxication therapy with AS (PHY/SCO/AS) offers the best protection against the soman-induced intoxication symptoms. In those cases where no therapy with AS was administered, the addition of SCO improved the protecting effect against soman-induced symptoms. This improvement has also been reported by others (17,19,32). SCO even offers a better result in counteracting soman or sarin-induced incapacitation than AS postintoxication therapy given to guinea pigs that were pretreated with a single dose of PHY (17). The positive effects in reducing postintoxication incapacitation of the addition of cholinolytics to PHY are also reflected in the shuttlebox data. A correlation is observed between the degree of intoxication symptoms and the performance in the shuttlebox. Such a correlation was described earlier in rats that only received therapy after soman intoxication (22). On the other hand, in the startle response task another picture appears. In all the situations an increase or a tendency towards an increase of the startle response was found. The addition of a single dose of AS significantly enhanced the effect on the startle reflex after soman intoxication in PHY- or PHY/SCO-pretreated animals. In a previous study it was clarified that direct effects on nicotinergic receptors were involved in the effects on the startle amplitude instead of AChE inhibition (24). This is in agreement with the results of Acri et al. (1), who have shown that nicotine causes a dose-dependent increase of the startle response. The results from a previous study indicate that a single dose of the muscarinic antagonist SCO antagonized the decreasing part of the bell-shaped dose–response curve of PHY on the startle reflex, leading to an increase of the response. This part of the curve of PHY on the startle reflex, leading to an increase of the response. This part of the curve could be mimicked by soman. This is corroborated by others: PHY, at higher dose levels (i.e., via AChE inhibition), activates neurons inhibiting the primary startle pathway. It appears that the pedunculopontine tegmental nucleus (PPTg) plays an important role in modulating sensorimotor gating by linking the ventral pallidum and the nucleus reticularis pontis caudalis, an obligatory part of the primary startle (7,29,30), via a direct, presumably muscarinic, cholinergic projection (16). This inhibitory circuit can be activated by acetylcholine agonists (16). Therefore, the increase of the startle response after a single injection with AS in PHY-pretreated and soman-intoxicated guinea pigs may be a result of inactivation of the inhibitory startle circuit that will lead to an increase of the startle response. For this reason, the animals of the PHY/SCO group seemed to be less affected than those of the PHY/SCO/AS group. Presumably, this strong effect on the startle amplitude may be less when the very high dose of AS is lowered compared with the realistic situation in humans (2 mg/70 kg). Furthermore, PHY administered in a single dose causing an increase of the startle amplitude also affected the VER (24). This effect on the N3 peak could, like the effect on the startle, not be counteracted by SCO. Therefore, these effects are most likely not the result of AChE inhibition. In this study, a comparable tendency was found on the VER in all the treatment groups. These findings strengthen the idea that other factors, like direct effects on nicotinergic receptors, play a role in the postintoxication incapacitation after soman. Interestingly, not only PHY exerts agonistic effects on nicotinergic receptors (2,27); soman also seems to exert such effects (3). Bakry et al. (3) reported that soman at micromolar concentrations can act as a partial agonist of the nicotinergic Ach receptor, and can induce receptor desensitization. This may influence the protecting efficacy against soman intoxication.

From the present experiments it may be concluded that the use of SCO as an adjunct pretreatment drug is not necessary to depress possible side effects, provided PHY pretreatment is given subchronically. However, in case soldiers are unable to use or are deprived from a postintoxication therapy, the addition of SCO to the PHY pretreatment can be life-saving. The pretreatment regime of PHY + SCO offers sufficient protection against lethality, but did not improve the postintoxication incapacitation when compared with a postintoxication therapy with AS after PHY or PHY + SCO pretreatment. These postintoxication effects could not completely be explained purely by AChE inhibition. Presumably, direct effects on nicotinergic receptors are also involved. In support of this opinion is the observation that after subchronic treatment with OPs muscarinergic and nicotinergic receptors become subsensitive to acetylcholine (5,6). Therefore, the treatment may further be improved by the addition of a nicotinergic antagonist.

In conclusion, subchronic treatment with the combination of PHY and SCO seems to be a better alternative for the current PYR pretreatment than PHY alone because it improves the protection against soman induced lethality in case a postintoxication therapy is not available. To further improve the efficacy of the treatment regime against postintoxication incapacitation, the addition of a nicotinic antagonist would be advisable. Such a treatment scenario will be examined in the future.

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