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Continuous Administration of Low Dose Rates of Physostigmine and Hyoscine to Guinea-pigs Prevents the Toxicity and Reduces the Incapacitation Produced by Soman Poisoning

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Abstract—A regime was developed, using mini-osmotic pumps, for the continuous subcutaneous administration of low doses of physostigmine (12·1, 9·7, 4·85 and 2·43 $\mu g\,h^{-1}$), in combination with hyoscine (1·94 or 0·39 $\mu g\,h^{-1}$), to guinea-pigs for up to 13 days. Physostigmine, in combination with hyoscine, inhibited plasma cholinesterase, and red blood cell and brain acetylcholinesterase, in a concentration-dependent manner, did not affect the normal growth rate of guinea-pigs, and produced no obvious signs of poisoning. A dose rate of 4·85 $\mu g\,h^{-1}$ physostigmine and 1·94 $\mu g\,h^{-1}$ hyoscine was required to inhibit red cell acetylcholinesterase by 30% and brain acetylcholinesterase by 5–15%, with an accompanying plasma hyoscine concentration of 700–850 $pg\,mL^{-1}$. There was an apparent decline in red cell acetylcholinesterase activity during the 13 days. Hyoscine levels were higher in the cholinergic-rich areas of the brain than in the plasma. Continuous pretreatment (1 or 6 days) with physostigmine (4·84 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$) provided complete protection against the lethal effects, and minimized the incapacitation and weight loss produced by soman at a dose equivalent to the LD99 value. Following soman challenge, guinea-pigs exhibited early signs of soman poisoning, but generally these signs of poisoning were minimal by 1–2 h. Extending the pretreatment time to 13 days protected 75% of the guinea-pigs against the lethal effects of soman poisoning. Red cell acetylcholinesterase activity, 24 h after soman poisoning, was higher following continuous pretreatment with physostigmine and hyoscine than after acute treatment with atropine.

The United Kingdom armed services are currently issued with a nerve agent pretreatment set (NAPS) containing pyridostigmine bromide tablets (30 mg to be taken 8-hourly). This pretreatment is supported by triple therapy based on atropine sulphate (2 mg), pralidoxime mesylate (500 mg) and the diazepam prodrug, avizafone (10 mg), only to be used in the event of organophosphate poisoning. Pyridostigmine pretreatment, supported by triple therapy or by atropine therapy, has been shown to protect guinea-pigs (Leadbeater et al 1985) and non-human primates (Dirnhuber et al 1979) against the lethal effects of soman poisoning, but was less effective in protecting against soman-induced incapacitation.

Previous studies have shown that the guinea-pig is the best non-primate-model for predicting the efficacy of treatments for organophosphate poisoning in primate species (Berry & Davies 1970; Gordon et al 1978; Dirnhuber et al 1979). Acute pretreatment with physostigmine and hyoscine protects against the lethal and incapacitating effects of soman poisoning in the guinea-pig (Leadbeater et al 1985; Lennox et al 1992) and the rhesus monkey (von Bredow et al 1991), and prevented the neurotransmitter changes produced by soman poisoning in the guinea-pig (Fosbraey et al 1991). However, the doses of physostigmine and hyoscine necessary to reduce the incapacitation produced by soman in the guinea-pig, inhibited the blood and brain acetylcholinesterase by some 57–67%.

The current pretreatment regime inhibits the red cell acetylcholinesterase by about 30% but does not inhibit brain acetylcholinesterase activity. Since physostigmine is able to cross the blood-brain barrier it inhibits brain acetylcholinesterase as well as red cell acetylcholinesterase. Thus, lower doses of physostigmine and hyoscine would need to be effective in preventing soman incapacitation in order that physostigmine and hyoscine could be considered as a possible treatment in man.

Following acute physostigmine and hyoscine pretreatment, the plasma concentrations of the pretreatment drugs would be declining during the soman challenge, whereas, if the drugs could be continuously administered, the tissue concentrations of the drugs should remain constant, and lower doses of the two drugs may prove effective.

The aim of this study was to establish a regime in the guinea-pig, for the continuous administration of low dose rates of physostigmine, in the presence of hyoscine, sufficient to inhibit red cell acetylcholinesterase by about 30%. The hyoscine dose rate should be the lowest which, in combination with physostigmine, protects all guinea-pigs against the lethal effects of soman poisoning.

Materials and Methods

Alzet pump preparation

Hyoscine and physostigmine (Sigma Chemical Co. Ltd, Poole, UK) were dissolved in sterilized saline (0·9% NaCl, w/v). Alzet miniature osmotic pumps (Charles River, UK) were used for a drug delivery over 1, 6 (model 2001) and 13 days (model 2002). Guinea-pigs did not receive the drugs during the initial 4 h after pump implantation, due to the start-up delay of the pump.

Alzet pump implant

Dunkin Hartley, male guinea-pigs, 168–290 g, were anaesthetized with halothane (Fluothane, ICI Ltd, 3% in oxygen), using a Halovet vaporizer (International Market Supply). A small area of the lower back was shaved, wiped with hibitane solution (5%) and sprayed with local anaesthetic (xylocaine, Astra Pharmaceuticals Ltd, Kings Langley, UK). A small incision was made in the back to create a channel just under the skin for the loaded Alzet pump. The incision was closed with two stitches. The guinea-pig was allowed to regain consciousness in a recovery cage, before being transferred to the animal house. A group of six animals was treated in the same way as the test animals, except that no Alzet pump was inserted (sham operation).

Experimental protocol

The starting weights of the guinea-pigs were chosen so that guinea-pigs should weigh 250–290 g on the day of the soman challenge; however, the weight of four out of six animals pre-treated for 13 days was 315–356 g on the day of soman challenge.

Blood samples were taken immediately before implantation of the pump and on days 1, 6 or 13 after pump implantation, for the measurement of acetylthiocholine hydrolase activities in plasma and red cells. Acetylthiocholine is hydrolysed by cholinesterase in the plasma and by acetylcholinesterase in red cells.

In preliminary studies, pretreatment with four different dose rates of physostigmine (12·1, 9·7, 4·85 and 2·43 $\mu g\,h^{-1}$) in combination with hyoscine (1·94 $\mu g\,h^{-1}$) was studied for its effect on blood and brain cholinesterase activities. Plasma and brain hyoscine levels were measured following physostigmine (4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 or 0·39 $\mu g\,h^{-1}$).

In protection studies, guinea-pigs were pretreated for 1, 6 or 13 days with physostigmine and hyoscine before subcutaneous injection of a soman dose equivalent to the LD99 (31·2 $\mu g\,kg^{-1}$). Physostigmine and hyoscine pretreatment was continuously administered during and after the soman dose.

To compare acetylcholinesterase activities 24 h after soman poisoning, with and without pretreatment, a group of guinea-pigs was given atropine (16 $mg\,kg^{-1}$) 15 min before soman to ensure that the guinea-pigs survived for 24 h after soman poisoning.

Experiments were performed according to the conditions of a Project Licence issued under the Animals (Scientific Procedures) Act 1986. Guinea-pigs were observed for the 6 h following soman challenge. Guinea-pigs were weighed daily, and any signs of poisoning (tremor, salivation, excessive chewing, preening, hyperactivity, unsteady gait or prostrate posture) due to pretreatment or soman were noted. These observations were subjective, and animals were recorded as having exhibited a particular sign of poisoning. The time when the animals no longer showed obvious signs of poisoning was recorded. Animals were considered as incapacitated when they showed signs of severe tremor, unsteady gait or prostrate posture. All animals were killed 24 h after soman administration, and blood and brain acetylcholinesterase activities determined.

Acetylcholinesterase assay

Blood samples (0·5 mL) were taken from the ear vein into tubes containing EDTA. Blood was separated into plasma and cells. The cells were washed once and made up to their original volume with saline (0·9% w/v). Resuspended cells, or plasma, were diluted 1 in 200 (v/v) with phosphate buffer (0·1 M, pH 8). Guinea-pigs were killed by cervical dislocation, and the brains dissected into six discrete regions (Glowinski & Iversen 1966). Brain samples were weighed and homogenized (100 mg wet weight tissue mL^{-1}), or (200 mg wet weight tissue mL^{-1}) for cortex, in phosphate buffer (0·1 M, pH 8). The homogenates were diluted 1 in 100 (v/v) or 1 in 200 (v/v) (cortex) with phosphate buffer. Samples were assayed at 30°C, with acetylthiocholine (1 mM), (BDH Chemicals Ltd, Poole, UK) as substrate, using a modification of the method of Ellman et al (1961). The change in absorbance was measured for 10 min (red cells), or for 6 min (plasma and brain).

Calculation of results

Enzyme activities were expressed as μmol acetylthiocholine hydrolysed min^{-1} (mL blood)$^{-1}$ or per 100 mg brain tissue. Enzyme activities for red cells and plasma, following implantation of the pumps, were also expressed as percent inhibition of the value obtained on day 0. A correction was made for any change in packed-cell volume caused by the washing procedure, and for any change in the whole-blood packed-cell volume that occurred over the experimental period. For the brain tissue, enzyme inhibition was measured as percent inhibition of the mean baseline activity for the corresponding tissue from 16–18 untreated animals (% mean control).

Hyoscine assay

Blood samples (1 mL) were taken from the ear vein into EDTA tubes. Plasma was separated from the red cells and frozen immediately at −70°C until assayed. Hyoscine concentrations were determined using a modified method of Metcalfe (1981), Muir & Metcalfe (1983) and Cintron & Chen (1987). Brain tissue was homogenized (100 $mg\,mL^{-1}$) in perchloric acid (0·1 M), and the homogenates were neutralized with sodium tetraborate (0·1 M). Samples were centrifuged and the supernatant diluted with phosphate buffer (0·01 M, pH 4). Hyoscine was extracted and concentrated from the brain and plasma samples with a Sep-Pak C18 column, and assayed with a radioreceptor assay, employing a receptor preparation from porcine brain.

Statistical analysis

Data were analysed for statistical significance using analysis of variance and Dunnett's test for acetylcholinesterase activities and an unpaired Mann Whitney U-test for percent inhibition. Weight change (%) was assessed by analysis of variance and Dunn's test and mortality rates were assessed using the Fischer exact test.

Results

Sham operation and bleeding

Red cell acetylcholinesterase activity increased during the 13 days after the sham operation, with the activities of the two guinea-pigs with the lowest acetylcholinesterase activities before surgery (0·55 and 0·64 $\mu mol\,min^{-1}\,mL^{-1}$) rising to

Table 1. The effect of continuous administration (13 days) of a combination of physostigmine (4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$) on the activities of red cell and brain regional acetylcholinesterase and plasma cholinesterase ($\mu mol\,min^{-1}\,mL^{-1}$) in the guinea-pig. Figures in brackets show the brain acetylcholinsterase inhibition as a percent of the mean control and plasma and red cell activities as percent own control.

	Pretreatment time (days)			
	0	1	6	13
n	16 or 18	6	9	8
Plasma	Own control	(30·2 ± 4·4)	(29·3 ± 3·5)	(34·9 ± 3·2)
Red cell	Own control	(26·1 ± 3·4)	(20·9 ± 2·4)	(0·24 ± 9·4)
Striatum	2·13 ± 0·05	2·02 ± 0·08	1·97 ± 0·07	1·9 ± 0·06
		(8·3 ± 5·1)	(7·4 ± 3·1)	(12·1 ± 4·1)
Hippocampus	0·61 ± 0·02	0·56 ± 0·02	**0·51 ± 0·02	0·55 ± 0·02
		(9·9 ± 2·8)	(16·2 ± 2·5)	(7·6 ± 4·9)
Cortex	0·57 ± 0·02	*0·48 ± 0·02	**0·44 ± 0·02	**0·44 ± 0·01
		(15·1 ± 3·3)	(25.1 ± 2·40)	(22·6 ± 2·1)
Mid brain	0·92 ± 0·02	**0·80 ± 0·03	**0·68 ± 0·02	**0·73 ± 0·009
		(14 ± 3·5)	(26·9 ± 1·9)	(20·8 ± 1·4)
Medulla pons	0·93 ± 0·02	**0·80 ± 0·03	**0·69 ± 0·01	**0·77 ± 0·02
		(13·4 ± 2·8)	(24·4 ± 1·3)	(16·3 ± 2·4)
Cerebellum	1·23 ± 0·06	1·15 ± 0·04	**0·93 ± 0·03	*1·02 ± 0·02
		(5·9 ± 3·6)	(23·8 ± 2·1)	(17·1 ± 1·4)

Results shown as mean ± s.e.m. Significance of difference from day 0, using analysis of variance and Dunnett's test, $*P < 0·05$, $**P < 0·01$.

139 and 138% of their day 0 values, respectively, by day 13. The packed cell volume did not change. Plasma cholinesterase activity increased by day 6, but not by day 13.

Physostigmine and hyoscine pretreatment

Blood and brain acetylcholinesterases were inhibited in a concentration-dependent manner. A physostigmine dose rate of 4·85 $\mu g\,h^{-1}$, in combination with hyoscine (1·94 $\mu g\,h^{-1}$), was required to produce a red cell acetylcholinesterase inhibition of 26%. Plasma cholinesterase was more inhibited, and regional brain acetylcholinesterase was similarly inhibited to red cell acetylcholinesterase (Table 1).

Hyoscine levels. The lower dose rate of hyoscine (0·39 $\mu g\,h^{-1}$), in combination with physostigmine (4·85 $\mu g\,h^{-1}$) produced plasma hyoscine levels of 525 $pg\,mL^{-1}$ on day 1, declining to 307 $pg\,mL^{-1}$ on day 6, whereas the higher dose rate of hyoscine (1·94 $\mu g\,h^{-1}$), in combination with physostigmine (4·85 $\mu g\,h^{-1}$) produced plasma hyoscine levels of 700–850 $pg\,mL^{-1}$ on days 1, 6 and 13. The higher dose rate of hyoscine produced regional differences in the levels of hyoscine in the brain. The hyoscine concentration was higher in the striatum, hippocampus and cortex than in the plasma. The hyoscine concentration in the striatum and hippocampus increased by day 13 (Table 2).

Growth rate. Physostigmine and hyoscine pretreatment did not affect the normal growth rate (3·4 ± 0·62%) of the guinea-pigs, except with physostigmine (9·7 $\mu g\,h^{-1}$) when there was a statistically significant lower weight gain by day 6.

Gross behaviour. There were no obvious signs of poisoning following the administration of physostigmine and hyoscine.

Physostigmine and hyoscine pretreatment and soman challenge

Blood cholinesterases. Pretreatment with physostigmine and hyoscine produced a similar pattern of inhibition of red cell acetylcholinesterase and plasma cholinesterase to those of the preliminary study (Tables 1, 3).

When guinea-pigs were pretreated for 6 days with physostigmine (4·85 $\mu g\,h^{-1}$) alone, or in combination with a lower dose rate of hyoscine (0·39 $\mu g\,h^{-1}$), plasma cholinesterase was significantly less inhibited than following pretreatment with physostigmine and the higher dose rate of hyoscine (Table 3).

Signs of poisoning. Following soman administration most guinea-pigs exhibited some signs of soman poisoning. Table 4 shows the signs of poisoning and the time course for recovery in guinea-pigs pretreated with physostigmine

Table 2. The effect of continuous administration (13 days) of a combination of physostigmine (4.85 $\mu g\,h^{-1}$) and hyoscine (1.94 $\mu g\,h^{-1}$) on the concentration of hyoscine in plasma ($ng\,mL^{-1}$) and brain regions ($ng\,g^{-1}$) of the guinea-pig.

	Pretreatment time (days)		
	1	6	13
Plasma	0·798 ± 0·087	0·700 ± 0·105	0·864 ± 0·0376
Striatum	5·09 ± 0·41	11·0 ± 1·87	*13·3 ± 2·17
Hippocampus	4·11 ± 0·57	3·52 ± 0·31	*6·91 ± 1·06
Cortex	7·00 ± 0·46	6·28 ± 0·58	6·09 ± 0·47
Mid brain	2·23 ± 0·35	1·41 ± 0·22	2·06 ± 0·32
Medulla pons	< 1·00	< 1·00	< 1·00
Cerebellum	1·46 ± 0·17	< 1.00	< 1.00

Results expressed as the mean ± s.e.m. (n = 4). Significance of difference from day 0, $*P < 0·05$ using analysis of variance and Dunnett's test.

Table 3. Percent inhibition of red cell acetylcholinesterase and plasma cholinesterase, 1, 6 or 13 days after continuous pretreatment with physostigmine (4·85 $\mu g\,h^{-1}$) and different dose rates of hyoscine immediately before challenge with a dose of soman equivalent to its LD99.

Pretreatment	Pretreatment time (days)					
	1		6		13	
	Red cell	Plasma	Red cell	Plasma	Red cell	Plasma
Physostigmine	*35·4	39·9	23·6	***50·3	15·5	*42·9
+hyoscine (1·94 $\mu g\,h^{-1}$)	±2·3	±1·6	±4·9	±1·8	±6·7	±2·6
Physostigmine	—	—	13·5	++37·1	—	—
+hyoscine (0·39 $\mu g\,h^{-1}$)			±4·7	±3·6		
Physostigmine alone	—	—	12·3	+++25·5	—	—
			±1·0	±2·1		

Results are expressed as mean ± s.e.m. (n = 8 or 9). Significance of difference from preliminary studies (Table 1) $*P < 0{\cdot}05$, $***P < 0{\cdot}001$ and significance of difference from physostigmine and hyoscine (1·94 $\mu g\,h^{-1}$) $^{++}P < 0{\cdot}01$, $^{+++}P < 0{\cdot}001$ using Mann Whitney U-tests.

(4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$) before soman challenge. Generally, these animals showed no obvious signs of poisoning by 2 h, whereas, guinea-pigs pretreated, either with physostigmine alone, or with physostigmine and hyoscine (0·39 $\mu g\,h^{-1}$), before soman challenge, still showed signs of poisoning at 4 h. Animals pretreated with the lower dose rate of physostigmine (2·43 $\mu g\,h^{-1}$) and hyoscine took more than 2 h to recover from soman challenge. Guinea-pigs, pretreated with hyoscine (1·94 $\mu g\,h^{-1}$) alone showed severe signs of soman poisoning.

Mortality. Continuous pretreatment with physostigmine (12·1, 9·8 or 4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$), for 1 or 6 days protected all guinea-pigs, and pretreatment for 13 days with physostigmine (4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$) protected 75% of guinea-pigs from the lethal effects of soman. Reducing the dose rate of hyoscine decreased the protection against soman lethality, as did pretreatment with physostigmine alone. Pretreatment with hyoscine alone was ineffective (Table 4). Reducing the dose rate of physostigmine, decreased the protection to 62·5 and 67% following 6 and 13 days pretreatment, respectively.

Growth rate. All guinea-pigs tended to lose weight following soman challenge (Table 4).

Cholinesterase activities 24 h after soman challenge. Red cell acetylcholinesterase activities, following pretreatment with physostigmine (4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$) and soman, were significantly higher 24 h after soman, than those for guinea-pigs treated with atropine and no pretreatment. Plasma cholinesterase activities were similar after pretreatment to those for the atropine-treated animals (Table 5). Similar results were obtained with the other dose rates of physostigmine and hyoscine (1·94 $\mu g\,h^{-1}$).

Brain acetylcholinesterase activities, following pretreatment with physostigmine (4·85 $\mu g\,h^{-1}$) and hyoscine (1·94 $\mu g\,h^{-1}$), and soman, were similar to those for atropine treatment (Table 5). Following 6 days pretreatment with physostigmine 4·85 $\mu g\,h^{-1}$ alone, or in combination with hyoscine (0·39 $\mu g\,h^{-1}$), and soman, red cell and brain acetylcholinesterase activities were generally higher than those after atropine-treatment and soman. Plasma cholinesterase activity appeared to increase as the hyoscine concentration decreased (Table 5).

Table 4. Signs of poisoning, weight change and mortality rates (24 h) for soman challenge (LD99) 31.2 $\mu g\,kg^{-1}$, 1, 6 or 13 days after continuous pretreatment with physostigmine (4·85 $\mu g\,h^{-1}$) and different dose rates of hyoscine.

	Time (min)	1		6				13
		Soman alone	Hyoscine 1·94 $\mu g\,h^{-1}$	Hyoscine 1·94 $\mu g\,h^{-1}$ alone	Hyoscine 1·94 $\mu g\,h^{-1}$	Hyoscine 0·39 $\mu g\,h^{-1}$	Hyoscine 0	Hyoscine 1·94 $\mu g\,h^{-1}$
Hyperactive		6/7	8/8	4/4	7/8	8/8	8/8	9/9
Chewing		6/7	6/8	0/4	5/8	6/8	8/8	7/9
Tremor		7/7	8/8	4/4	6/8	8/8	8/8	9/9
Unsteady gait		0/7	1/8	0/4	1/8	0/8	1/8	1/9
Prostrate		6/7	4/8	4/4	4/8	5/8	5/8	5/9
Number showing	30	7/7	8/8		6/8	8/8	8/8	9/9
obvious signs	60	7/7	2/8		3/8	6/8	7/8	6/9
of poisoning	120	7/7	0/8		1/8	5/8	5/8	3/9
	240		0/8		1/8	4/7	3/6	2/9
	360		0/8		1/8	4/7	1/4	1/8
% weight loss/gain		—	−2·1	—	−4·4	−5·1	0·9	*−4·1
			±0·8		±2·7	±3·9	±1·49	±1·6
Mortality		7/7	0/8	4/4	0/8	1/8	*4/8	2/9
		(< 4 h)		(< 30 min)				

Results are expressed as mean ± s.e.m. For weight loss significant difference from control group $*P < 0{\cdot}05$ using analysis of variance and Dunn's test, for mortality rates significant difference from physostigmine and hyoscine pretreatment (day 1).
$*P < 0{\cdot}05$ using Fischer exact test.

Table 5. Acetylcholinesterase activities (μmol min^{-1} mL^{-1}) of blood and brain areas 24 h after soman (LD99) challenge, following treatment with atropine (15 min) or pretreatment with physostigmine (4·85 μg h^{-1}) and different dose rates of hyoscine (1, 6 or 13 days). Figures in brackets show percent activity of acetylcholinesterase.

	0	1	6			13
	Atropine 16 mg mg^{-1}	Hyoscine 1·94 μg h^{-1}	Hyoscine 1·94 μg h^{-1}	Hyoscine 0·39 μg h^{-1}	Hyoscine 0	Hyoscine 1·94 μg h^{-1}
Plasma	0·53 ± 0·05 (28·9 ± 1·7)	0·53 ± 0·03 (30·3 ± 1·1)	0·46 ± 0·02 (21·1 ± 1·3)	0·53 ± 0·04 (30·1 ± 1·6)	0·64 ± 0·04 (34·6 ± 2·8)	0·50 ± 0·04 (25·6 ± 1·6)
Red cell	0·06 ± 0·013 (6 ± 1·4)	*0·13 ± 0·012 (11·3 ± 1·3)	*0·13 ± 0·009 (14·1 ± 1·5)	**0·16 ± 0·02 (16·2 ± 1·9)	**0·15 ± 0·013 (16·2 ± 1·9)	*0·13 ± 0·01 13·8 ± 0·9
Striatum	0·38 ± 0·04 (17·9 ± 2)	0·49 ± 0·05 (22·9 ± 2·4)	0·49 ± 0·05 (23·1 ± 2·5)	*0·65 ± 0·09 (26·7 ± 4·2)	**0·75 ± 0·18 (35·2 ± 8·3)	0·51 ± 0·04 (23·9 ± 1·9)
Hippocampus	0·13 ± 0·008 (21·6 ± 1·3)	0·15 ± 0·035 (24·2 ± 2·1)	0·13 ± 0·011 (20·8 ± 1·9)	0·18 ± 0·016 (25·3 ± 2·2)	**0·21 ± 0·019 (34·2 ± 3·1)	0·14 ± 0·008 (22·4 ± 1·2)
Cortex	0·11 ± 0·006 (18·7 ± 1·06)	0·135 ± 0·012 (22·7 ± 1·6)	0·106 ± 0·009 (18·5 ± 1·7)	0·143 ± 0·016 (21·8 ± 2·8)	0·143 ± 0·02 (25·1 ± 4·2)	0·105 ± 0·008 (21·2 ± 1·5)
Mid brain	0·191 ± 0·008 (20·6 ± 0·8)	0·197 ± 0·008 (21·2 ± 0·9)	0·184 ± 0·008 (19·8 ± 0·84)	0·199 ± 0·017 (21·6 ± 1·7)	0·223 ± 0·025 (26·4 ± 1·2)	0·191 ± 0·007 (20·5 ± 0·8)
Medulla pons	0·189 ± 0·011 (24·9 ± 2·5)	0·247 ± 0·012 (26·7 ± 1·3)	0·206 ± 0·013 (22·3 ± 1·4)	*0·279 ± 0·023 (26·6 ± 2·3)	*0·298 ± 0·039 (32·2 ± 4·2)	0·233 ± 0·008 (25·2 ± 0·9)
Cerebellum	0·243 ± 0·02 (19·8 ± 1·7)	0·299 ± 0·02 (24·4 ± 1·9)	0·259 ± 0·02 (21·1 ± 1·1)	0·288 ± 0·03 (23·3 ± 2·8)	0·317 ± 0·03 (25·9 ± 2·6)	0·255 ± 0·02 (19·4 ± 2·1)

Results are expressed as mean ± s.e.m. (n = 7 or 8). Significant difference from atropine-treated group *$P < 0.05$, **$P < 0.01$ using analysis of variance and Dunnett's test.

Discussion

This study has shown that the continuous administration of physostigmine and hyoscine to guinea-pigs for up to 13 days, inhibits plasma cholinesterase, and red cell and brain acetylcholinesterase, in a concentration-dependent manner, does not affect the normal growth rate of guinea-pigs and produces no obvious signs of poisoning.

Continuous pretreatment with physostigmine and hyoscine at a dose rate to inhibit red cell acetylcholinesterase by only 30% affords good protection against the lethal effects, and minimizes the signs of poisoning and weight loss, produced by soman.

The decrease in the inhibition of red cell acetylcholinesterase activity after 13 days pretreatment may be due to changes in the basal levels of acetylcholinesterase. Results from the sham-operation group showed that the red-cell acetylcholinesterase activity in three of the six guinea-pigs increased over the 13 days. Another possible explanation for the decrease in red cell-acetylcholinesterase inhibition following physostigmine and hyoscine administration over 13 days is that the dose of drug per unit weight decreased, because the drug was applied at a constant rate while the weight of the guinea-pig was increasing. However, growth rate did not appear to reduce the percent inhibition of plasma cholinesterase or decrease the plasma hyoscine levels.

Hyoscine levels, following pretreatment with physostigmine and hyoscine, were higher in the cholinergic-rich areas of the brain than in the plasma. The increase in hyoscine levels in the striatum and hippocampus may be due to a change in receptor-binding affinity, or to an increase in receptor density. Other workers have shown that continuous administration of trihexyphenidyl decreased the binding affinity and increased the binding density of [^{3}H]QNB and [^{3}H]pirenzepine; however, coadministration of trihexiphenidyl and physostigmine produced no effect on binding in the guinea-pig striatum (Lim et al 1991).

Pretreatment with physostigmine and hyoscine, at a dose to inhibit red cell acetylcholinesterase activity by 30%, did not prevent the early signs of soman poisoning; however, the severity and the duration of the signs of poisoning were reduced. This regime protected all animals from the lethal effects of soman following six days pretreatment. Other workers have reported similar findings with higher dose rates of physostigmine in guinea-pigs (Lim et al 1989a, b; Anderson et al 1991).

The loss of some protection against soman lethality following 13 days pretreatment may be due either to the higher weight of animals in this group, or to the decrease in red cell acetylcholinesterase inhibition produced by the pretreatment.

The higher levels of red cell acetylcholinesterase activity, 24 h after soman challenge, following physostigmine and hyoscine pretreatment, than after atropine treatment, demonstrate that the pretreatment has protected a portion of the acetylcholinesterase from inhibition by soman. The enzyme was protected even though the pretreatment drugs were continuously administered during and after the soman challenge.

The lack of difference in regional brain acetylcholinesterase activities, following physostigmine and hyoscine pretreatment and those following atropine treatment is probably due in part to the method of calculating brain acetylcholinesterase activities. The inhibition produced by soman is irreversible, so the particular guinea-pig's baseline acetylcholinesterase activity cannot be calculated. There is a wide variation in regional brain acetylcholinesterase activities amongst individuals, and therefore the small changes in enzyme activities that are probably due to the pretreatment are difficult to demonstrate.

The differences in cholinesterase levels observed following pretreatment for six days with physostigmine alone, or with physostigmine and hyoscine, showed that, as the hyoscine dose rate decreased, there was an apparent increase in brain acetylcholinesterase and plasma cholinesterase activities 24 h after soman poisoning. There was also a dose-

dependent effect of hyoscine on the inhibition of plasma cholinesterase before soman poisoning. The mechanism by which hyoscine affects the activities of brain and plasma cholinesterases is unclear. However, the benefits of pretreatment with physostigmine and the higher dose rate of hyoscine in protecting against the lethal and incapacitating effects of soman are in no doubt.

It is concluded that continuous administration of low dose rates of physostigmine and hyoscine protect against the lethal effects, and minimize the incapacitation produced by soman. Also, the continued administration of the pretreatment drugs during and after soman challenge does not exacerbate the effects of soman and may prove beneficial as an addition to therapy. Therefore, it is likely that continuous administration of low dose rates of physostigmine and hyoscine will also protect the non-human primate against the effects of soman poisoning. These low dose rates of physostigmine and hyoscine may be acceptable as a pretreatment in man, and, if so, should prove effective in protecting against the lethal and the incapacitating effects of nerve-agent poisoning in this species, too.

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