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TITLE

IN VITRO OXIME-INDUCED REACTIVATION OF VARIOUS MOLECULAR FORMS OF SOMAN-INHIBITED ACETYLCHOLINESTERASE IN STRIATED MUSCEL FROM RAT, MONKEY AND HUM

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In vitro oxime-induced reactivation of various molecular forms of soman-inhibited acetylcholinesterase in striated muscle from rat, monkey and human

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Abstract. The purpose of this study was to compare the in vitro reactivation of the various molecular forms of soman-inhibited acetylcholinesterase by oximes such as HI-6, toxogonin and PAM, in striated muscle tissue from three species-rat, monkey and human. To simulate the various in vivo conditions the oxime was present either 5 min before and after (Pre-Post) or 5 min after (Post) exposure to the nerve agent soman. In the Pre-Post mode the oxime effects would result from a combination of not only shielding of acetylcholinesterase from soman inhibition but also from immediate reactivation of soman-inhibited acetylcholinesterase. In the Post experimental group the increase in soman-inhibited acetylcholinesterase activity was due to reactivation. HI-6 (Pre-Post) increased significantly the activity of soman-inhibited acetylcholinesterase in the rat, human and monkey muscle. HI-6 (Post) was a highly effective reactivator of soman-inhibited acetylcholinesterase in the rat muscle and moderately so in the human and monkey muscle. Toxogonin (Pre-Post) and toxogonin (Post) were effective in increasing soman-inhibited acetylcholinesterase activity in rat muscle but were relatively ineffective in the human and monkey muscle. PAM (Pre-Post) and PAM (Post) were ineffective in increasing soman-inhibited acetylcholinesterase activity in muscle from all species examined. Effectiveness of oxime-induced reactivation of soman-inhibited acetylcholinesterase could be estimated from the total acetylcholinesterase activity which appears to reflect the results found with the individual molecular forms of acetylcholinesterase. In addition, SAD-128, a non-oxime bispyridinium compound, appeared to enhance significantly the HI-6 induced reactivation of soman-inhibited acetylcholinesterase in human but not rat striated muscle.

Key words: Oxime – Soman – Acetylcholinesterase – Striated muscle – Rat – Human – Monkey

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Introduction

Organophosphate nerve agents such as sarin, tabun and VX are generally considered to be irreversible inhibitors of acetylcholinesterase which phosphorylate the active site of acetylcholinesterase. This biochemical lesion can be influenced by the use of an acetylcholinesterase oxime reactivator such as PAM (pyridine 2-aldoxime chloride) or toxogonin (1,1′-[oxybis(methylene)]bis[4-hydroxyimino)-methyl]pyridinium dibromide) which dephosphorylates the active site. However, in the case of soman (pinacolyl methylphosphonofluoridate) poisoning these oximes are not effective. The bispyridinium oxime HI-6, ([[[(4-aminocarbonyl)pyridinio]methoxy]methyl]-2-[(hydroxyimino)-methyl]pyridinium dichloride monohydrate) is the most potent reactivator of "unaged" (Berends 1987) soman-inhibited acetylcholinesterase (DeJong and Wolring 1980, 1984; Boskovic 1981; Clement 1983). Soman-inhibited acetylcholinesterase undergoes an ageing process by which it is converted to a form that cannot be reactivated by oximes (Berends, 1987). In vivo, the combination of HI-6 plus atropine sulfate has proved to be an efficient treatment regimen for all nerve agents (Clement 1981, 1983; Hamilton and Lundy 1989; VanHelden et al. 1992).

The hydrolytic enzyme acetylcholinesterase inactivates the neurotransmitter acetylcholine at cholinergic synapses. This polymorphic enzyme is found in many neuronal and non-neuronal tissues and is usually composed of several molecular forms (Massoulie and Bon 1982; Inestrosa and Perelman 1989). Two general classes of acetylcholinesterase are called globular (G) and asymmetric (A). The globular class has three forms designated G1, G2 and G4, based on sedimentation analysis, composed of a monomer, dimer and tetramer, respectively, while the asymmetric class has three forms called A4, A8 and A12 which consist of aggregates of one, two and three tetramers, respectively, with a collagen tail attached. Three molecular forms of acetylcholinesterase predominate in striated muscle with sedimentation coefficients of 4S, 10S and 16S which correspond to the G1, G4 and A12 forms, respectively.

Table 1. Protocol and timing sequence[1]

Timing of Transfers (min)	Control	Soman Control	Oxime (Pre-Post)[b]	Oxime (Post)	SAD-128 (Pre-Post) + HI-6 (Post)	SAD-128 (Pre-Post)
0	Ringer	Ringer	Oxime	Ringer	SAD-128	SAD-128
5		Soman	Soman + Oxime	Soman	Soman + SAD-128	Soman + SAD-128
10		Wash with Ringer	Wash with Oxime	Wash with Oxime	Wash with HI-6	Wash with SAD-128
13			Oxime	Oxime	HI-6	SAD-128
43	Wash with Ringer	Wash with Ringer	Wash with Ringer	Wash with Ringer	Wash with Ringer	Wash with Ringer
48	Homogenize	Homogenize	Homogenize	Homogenize	Homogenize	Homogenize

[a] The concentration of soman was 180 nM. The concentration of the oximes (HI-6, toxogonin and PAM) or SAD-128 was 0.5 mM. The various compounds were all made up in Ringer solution. The timing of transfers was the time at which the transfers into the next solution in the column took place. The start time was "0" min. At the end of the 48 min incubation sequence the tissue was homogenized

[b] Throughout this study "Pre-Post" describes the situation where the oxime (or SAD-128) was present before during and after the exposure to soman. "Post" describes the situation where the oxime was present only after exposure to soman

SAD-128 (1,1′-oxydimethylene bis/4-tert-butylpyridinium chloride) is a bis-pyridinium compound with no oxime moiety, which reversibly inhibited acetylcholinesterase and retards the rate of ageing of soman-inhibited acetylcholinesterase (Harris et al. 1978; Schoene 1978; Grubic and Tomazic 1989; Stalc and Sentjurc 1990).

The purpose of this study was to compare the reactivation of the individual molecular forms of soman-inhibited acetylcholinesterase by oximes such as HI-6, toxogonin and PAM, in muscle tissue from three different species-rat, monkey and human. The effect of SAD-128, a non-oxime bispyridinium compound, on the effectiveness of HI-6 was also evaluated.

Materials and methods

Protocol

The experimental design (Table 1) used in these experiments was similar to that described previously (Grubic and Tomazic 1989) and was such that it would test the effectiveness of the oximes and simulate in vivo conditions, i.e. either the oxime was present before and after the agent or the oxime was present after exposure to the agent. The various experimental groups were as follows.

Control. The tissue which was taken through the entire protocol but was not exposed to either oxime or soman, and thus represented 100% acetylcholinesterase activity.

Soman control, acetylcholinesterase activity following treatment with soman only.

Pre-Post, the oxime or SAD-128 was present before during and after soman. The acetylcholinesterase activity would be due to the oxime shielding of the enzyme to inhibition by soman and also due to immediate reactivation of the acetylcholinesterase by the oxime.

Post, the oxime was only present after the nerve agent. Acetylcholinesterase activity would result from the reactivation of soman-inhibited acetylcholinesterase.

The effect of SAD-128 (Pre-Post) and the effect of SAD-128 (Pre-Post) and HI-6 (Post) on the activity of soman-inhibited acetylcholinesterase was also evaluated.

Tissue sources

Rats. Male Sprague-Dawley rats obtained from Charles River Canada, St Constant, Quebec, were used in this study. The animals were kept in the vivarium at Defence Research Establishment Suffield for at least 1 week prior to experimentation. The animals were allowed access to food and water ad libitum. The room temperature was 21–22°. The photoperiod was 12 h with the lights on at 0700 hours. All experiments were performed using fresh rat tissue. Rats were decapitated and exsanguinated. The diaphragm was extirpated, rinsed in ice cold saline and sliced into right and left hemidiaphragms (approximately 300 mg). The central tendinous portion of the diaphragm was removed and discarded.

Monkeys. Samples of skeletal muscle from 4 to 6-year-old male and female monkeys (Cynomolgus, Indonesian origin) were obtained from Charles River Primates Corp. (Houston, Tex.). The muscle was shipped frozen on dry ice. The muscle sample was thawed and sliced into pieces of approximate size as used in the rat experiments.

Human. Skeletal muscle tissue was obtained from patients undergoing either hip replacement or gall bladder surgery. The harvesting of the tissue was approved by the Tissue Committee at The Medicine Hat Regional Hospital and the patients all signed an informed consent form. Due to the irregularity of the supply the tissue was harvested and kept soaked in 0.9% ice cold saline and transported to Defence Research Establishment Suffield where it was stored frozen at –80° C. On the day of the experiment the tissue was thawed and cut into the slices approximating in size and thickness those used in the rat experiments.

All experiments with human and monkey tissue were carried out in a biohazard hood and all tissue and fluid was decontaminated in bleach and incinerated upon completion of the experiment.

Tissue preparation

The tissue slices were transferred to a small petri dish containing Ringer solution on an orbital shaker platform. Incubations were performed at room temperature and an orbital shaking rate of 85 rpm. The various solutions were placed in a series of petri dishes according to the protocol of Grubic and Tomazic (1989) as outlined in Table 1. The tissue was transferred, using forceps, to different solutions and during the transfer the tissue was blotted on filter paper to remove excess fluid. At the end of the incubation period the tissue was transferred to a centrifuge tube containing 1 ml homogenization buffer (0.01 M TRIS, 0.05 M $MgCl_2$, 1.0 M NaCl and 1% (w/w) Triton x-100)/100 mg tissue. The inclusion of Triton x-100 resulted in 99.5% of cholinesterase activity present in the supernatant (Shih 1993). The tissue was homogenized at 4° C, using a Polytron homogenizer (Brinkman) at set-

Table 2. Activities of the molecular forms of acetylcholinesterase in rat diaphragm following in vitro exposure to soman and various oxime reactivators[a]

	Acetylcholinesterase activity (nmol/ml per min)				
	Molecular forms				
Treatment group	Total	4S	10S	16S	*N*
Untreated control	125.03 ± 13.87[b] 100	48.45 ± 6.47 100	54.37 ± 7.20 100	22.21 ± 3.75 100	6
Soman control	31.39 ± 9.26 25	12.07 ± 3.96 25	11.99 ± 3.34 22	7.33 ± 2.26 33	6
HI-6 (Pre-Post)	94.88 ± 15.30[c] 76	31.13 ± 6.17[c] 64	45.21 ± 10.00[c] 83	18.54 ± 1.80[c] 84	7
HI-6 (Post)	74.81 ± 10.11[c] 60	23.67 ± 3.88[c] 49	35.39 ± 6.34[c] 65	15.74 ± 1.93[c] 71	7
Toxogonin (Pre-Post)	79.67 ± 10.69[c] 64	30.15 ± 3.74[c] 62	33.52 ± 9.98[c] 62	16.00 ± 1.71[c] 72	6
Toxogonin (Post)	62.97 ± 6.03[c] 50	24.50 ± 2.96[c] 51	23.76 ± 4.53 44	14.71 ± 2.00[c] 66	6
PAM (Pre-Post)	32.42 ± 5.84 26	11.80 ± 1.68 24	13.41 ± 4.28 25	7.20 ± 1.11 32	6
PAM (Post)	34.43 ± 7.99 28	12.50 ± 2.50 26	14.14 ± 4.12 26	7.78 ± 1.84 35	6
ANOVA $F(7,42)$ = $p <$	62.24 0.01	52.51 0.01	33.47 0.01	42.12 0.01	

[a] Rat diaphragm was incubated at room temperature using the experimental protocols outlined in Table 1

[b] Mean ± SD with the % control value underneath in boldface

[c] Significantly different from the soman control group $p < 0.01$

ting 6 for approximately 30 s. The homogenate was centrifuged (Beckman J2-21 M) at 20000 *g* for 20 min at 4° C. The supernatant was removed and clarified further by loading into 3 ml syringes and filtering through 0.45 µm filters (Acrodisk, Gelman Sciences, Ann Arbor, Mich.). These supernatants (200 µl) were then loaded, in duplicate, onto sucrose gradients.

The sucrose density gradients were prepared and centrifuged as described previously (Clement et al. 1991).

Acetylcholinesterase

Each fraction was analyzed, in duplicate, for acetylcholinesterase activity by the method of Ellman (Ellman et al. 1961) using a microplate assay. Each fraction contained ISO-OMPA (10 µM) to selectively inhibit pseudocholinesterase activity. All manipulations of the microplate were performed using a Biomek 1000 Automated Laboratory Workstation (Beckman Instruments). The optical density was read at a wavelength of 405 nm using a SLT 400 AT (SLT-Labinstruments) microplate reader.

Data analysis.

The acetylcholinesterase activity for the various fractions was determined, plotted and the area under the curve (AUC) of the various molecular forms was determined using a Lotus 123 template. The 4S form included fractions 1–14, 10S form included fractions 15–23 and 16S form included fractions 24–32 for rat and human tissue and 1–12, 13–23 and 24–32 for 4S, 10S and 16S forms, respectively, in monkey tissue. Total acetylcholinesterase activity was the sum total of the activity of all of the fractions. All fractions were corrected for nonenzymatic hydrolysis using the activity of fractions from a run where extraction buffer (no tissue) was layered on the sucrose gradient, centrifuged, fractionated and acetylcholinesterase activity determined.

The data were analyzed by one-way ANOVA and when the result was statistically significant the rigorous multiple comparison test of Scheffe (Ferguson 1971) was used to determine individual differences. A difference with $p < 0.05$ was considered statistically significant.

Materials Soman, HI-6, toxogonin, PAM and SAD-128, prepared at Defence Research Establishment Suffield, were in excess of 99% pure. Other substances used were obtained from various commercial sources.

Results

Experiments with HI-6, toxogonin and PAM

Rat. In vitro exposure (Table 1) of the rat diaphragm tissue to soman (180 nM) reduced significantly ($p < 0.01$) the total acetylcholinesterase activity and activity of the various molecular forms (Table 2). HI-6 (Pre-Post) resulted in higher acetylcholinesterase activity, although not significantly higher, than HI-6 (Post). Similar results were found for toxogonin. Even though the acetylcholinesterase activity after HI-6 exposure tended to be higher than that following toxogonin exposure, the difference between the two oximes was not statistically significant. The acetylcholinesterase activity did not increase with any of the PAM treatments (Table 2).

Monkey. In vitro exposure of monkey skeletal muscle to soman (180 nM) significantly reduced acetylcholinesterase

Table 3. Activities of the molecular forms of acetylcholinesterase in monkey muscle following in vitro exposure to soman and various oxime reactivators[a]

	Acetylcholinesterase activity (nmol/ml per min)				
	Molecular forms				
Treatment group	Total	4S	10S	16S	*N*
Untreated control	70.17 ± 14.83[b] 100	22.07 ± 7.57 100	13.46 ± 2.35 100	34.64 ± 7.88 100	12
Soman control	33.15 ± 5.73 47	8.60 ± 2.15 39	6.89 ± 1.25 51	17.66 ± 2.81 51	12
HI-6 (Pre-Post)	66.20 ± 15.11[c] 94	20.62 ± 7.82[c] 93	13.07 ± 2.53[c] 97	32.51 ± 6.74[c] 94	12
HI-6 (Post)	41.10 ± 4.01 59	10.82 ± 2.24 49	8.08 ± 1.63 60	22.20 ± 3.37 64	12
ANOVA $F(3,43)$ $p <$	32.33 0.01	17.34 0.01	33.85 0.01	25.09 0.01	
Untreated control	79.87 ± 15.19 100	27.76 ± 11.32 100	15.80 ± 3.07 100	36.32 ± 10.09 100	4
Soman control	28.02 ± 6.56 35	7.97 ± 2.47 29	6.03 ± 1.51 38	14.02 ± 2.88 39	4
Toxogonin (Pre-Post)	26.41 ± 5.29 33	8.21 ± 2.40 30	5.77 ± 1.09 37	12.44 ± 1.90 34	4
Toxogonin (Post)	27.11 ± 4.68 34	8.78 ± 2.45 32	5.15 ± 1.86 33	13.17 ± 1.05 36	4
PAM (Pre-Post)	23.93 ± 6.05 30	8.29 ± 1.74 30	4.67 ± 1.78 30	10.97 ± 2.59 30	4
PAM (Post)	26.82 ± 8.00 34	8.45 ± 4.45 30	5.64 ± 1.60 36	12.73 ± 2.23 35	4
ANOVA $F(5,18) =$ $p <$	27.01 0.01	8.94 0.01	19.65 0.01	17.87 0.01	

[a] Tissue was processed as outlined in Fig. 1 except that the quantitation of the various molecular forms was as follows: fractions 1–12 = 4S, fractions 13–19 = 10S and fractions 29–32 = 16S

[b] Mean ± SD with the % control value underneath in boldface

[c] Significantly different from the soman control group, $p < 0.01$. All other values except these were significantly different from the untreated control group

activity in all fractions (Table 3). Only HI-6 was effective in providing an increase in the total activity of soman-inhibited acetylcholinesterase and in the activity of the various molecular forms. HI-6 (Pre-Post) treatment produced the highest acetylcholinesterase activity; HI-6 (Post) was significantly less effective. The activity of soman-inhibited acetylcholinesterase did not significantly increase with any of the treatments with PAM or toxogonin.

Human. As was found with the other species, in vitro exposure of human skeletal muscle to soman (180 nM) significantly reduced acetylcholinesterase activity in all fractions (Table 4). HI-6 (Pre-Post) resulted in the highest acetylcholinesterase activity whereas HI-6 (Post) was significantly less effective. Toxogonin (Pre-Post), toxogonin (Post), PAM (Pre-Post) and PAM (Post) always produced small, but insignificant, increases in activity (Table 4).

Experiments with SAD-128

The effect of SAD-128 alone (Pre-Post) and the effect of SAD-128 (Pre) followed by HI-6 (Post) on the activity of soman-inhibited acetylcholinesterase was evaluated. SAD-128 alone (Pre-Post) resulted in significantly greater total acetylcholinesterase activity (Table 5) but did not produces statistically significant increase in the activity of any of the individual molecular forms. The results of treatment of SAD-128 (Pre) and HI-6 (Post) (70% activity; Table 5) were not significantly different from those obtained with HI-6 alone (Post) (60% activity; see Table 2).

There appeared to be a larger degree of variability when SAD-128 was included in the human muscle experiments. In the human muscle (Table 6), SAD-128 alone (Pre-Post) did not produce a statistically significant increase in acetylcholinesterase activity but did enhance the efficacy of HI-6 (Post), e.g. HI-6 (Post), following SAD-128 (49%; Table 6) was significantly different from HI-6 (Post) (19%; see Table 4).

Discussion

The effects of oximes on soman-inhibited acetylcholinesterase could be estimated from the total acetylcholinesterase activity which appears to reflect the results found

Table 4. Activities of the molecular forms of acetylcholinesterase in human muscle following in vitro exposure to soman and various oxime reactivators[a]

	Acetylcholinesterase activity (nmol/ml per min)				
	Molecular forms				
Treatment group	Total	4S	10S	16S	*N*
Untreated control	456.71 ± 150.53[b] 100	271.86 ± 111.37 100	88.97 ± 26.71 100	95.88 ± 32.59 100	12
Soman control	45.08 ± 17.11 10	19.62 ± 9.09 7	11.77 ± 5.22 13	13.76 ± 5.66 14	11
HI-6 (Pre-Post)	294.98 ± 102.84[c] 65	180.47 ± 88.30[c] 66	57.21 ± 12.98[c] 64	57.30 ± 17.98[c] 60	8
HI-6 (Post)	85.27 ± 26.65 19	46.37 ± 16.71 17	18.96 ± 8.13 21	19.93 ± 4.69 21	8
Toxogonin (Pre-Post)	76.83 ± 32.21 17	38.27 ± 18.41 14	18.81 ± 8.09 21	19.75 ± 7.12 21	7
Toxogonin (Post)	93.94 ± 55.28 21	41.33 ± 25.73 15	24.56 ± 15.06 28	28.05 ± 18.11 29	7
PAM (Pre-Post)	81.69 ± 45.76 18	38.10 ± 24.53 14	20.85 ± 12.15 23	22.74 ± 10.38 24	6
PAM (Post)	64.97 ± 33.84 14	27.88 ± 16.65 10	17.86 ± 9.16 20	19.22 ± 9.16 20	6
ANOVA $F(7,57)$ = $p <$	34.04 0.01	24.10 0.01	33.70 0.01	27.12 0.01	

[a] Tissue was processed as outlined in Fig. 1 at room temperature
[b] Mean ± SD, with the % control value underneath in boldface
[c] Significantly different from the soman control group ($p < 0.01$). All the other values were significantly different from the untreated control group ($p < 0.01$)

with the individual molecular forms of acetylcholinesterase (Fig. 1). Generally, there did not appear to be any selectivity with regards to the activity of the various molecular forms of soman-inhibited acetylcholinesterase following treatment with the various oximes in the various species. This was particularly evident in the case of the rat diaphragm. Similar results were reported by Clement et al. (1991) for mice.

The results of the present study are similar to those of Grubic and Tomazic (1989) with respect to the reactivation of soman-inhibited acetylcholinesterase in the rat and human skeletal muscle and extend the observations of Grubic and Tomazic to include the effects of three different oximes on soman-inhibited acetylcholinesterase in three different species. HI-6 (Pre-Post) increased significantly the activity of soman-inhibited acetylcholinesterase in the

Table 5. Effect of SAD-128 alone and combined with HI-6 on the activities of the molecular forms of acetylcholinesterase from rat muscle following in vitro exposure to soman[a]

	Acetylcholinesterase activity (nmol/ml per min)				
	Molecular forms				
Treatment group	Total	4S	10S	16S	*N*
Untreated control	125.03 ± 13.87[b] 100	48.45 ± 6.47 100	54.37 ± 7.20 100	22.21 ± 3.75 100	6
Soman control	31.39 ± 9.26 25.11	12.07 ± 3.96 24.92	11.99 ± 3.34 22.05	7.33 ± 2.26 33.00	6
SAD-128 (Pre-Post)	63.39 ± 14.79[c] 50.70	20.13 ± 4.62 41.55	30.31 ± 9.15 55.75	12.95 ± 3.55 58.32	8
SAD-128 (Pre-Post) + HI-6 (Post)	87.96 ± 29.15[d] 70.35	26.69 ± 7.28[d] 55.09	43.61 ± 19.15[d] 80.22	17.65 ± 4.50[d] 79.49	8
ANOVA $F(3,24)$ = $p <$	25.94 0.01	44.16 0.01	14.29 0.01	18.52 0.01	

[a] The protocol was as outlined in Fig. 1
[b] Mean ± SD with the % control values underneath in boldface
[c] Significantly different from soman control $p < 0.05$
[d] Significantly different from soman control $p < 0.01$

Table 6. Effect of SAD-128 alone and combined with HI-6 on the activities of the molecular forms of acetylcholinesterase in human muscle following in vitro exposure to soman[a]

	Acetylcholinesterase activity (nmol/ml per min)				
	Molecular forms				
Treatment group	Total	4S	10S	16S	*N*
Untreated control	456.71 ± 150.53[b] 100	271.86 ± 111.37 100	88.97 ± 26.71 100	95.88 ± 32.59 100	12
Soman control	45.08 ± 17.11 10	19.62 ± 9.09 7	11.77 ± 5.22 13	13.76 ± 5.66 14	11
SAD-128 (Pre-Post)	112.70 ± 41.40 25	61.43 ± 24.70 23	25.97 ± 9.95 29	25.30 ± 8.77 26	7
SAD-128 (Pre-Post) + HI-6 (Post)	223.96 ± 59.83[c] 49	128.30 ± 43.34[c] 47	45.72 ± 12.79[c] 51	49.94 ± 16.38[c] 52	6
ANOVA $F(3,32)$ = $p <$	41.41 0.01	28.98 0.01	42.44 0.01	33.97 0.01	

[a] Tissue was processed as outlined in Fig. 1
[b] Mean ± SD with the % control value underneath in boldface
[c] Significantly different from the soman control group ($p < 0.01$). All the values were significantly different from the untreated control group ($p < 0.01$)

rat, human and monkey muscle (Fig. 1). HI-6 (Post) was a highly effective reactivator of soman-inhibited acetylcholinesterase in the rat muscle and moderately so in the human and monkey muscle. Toxogonin (Pre-Post) and toxogonin (Post) effectively reactivated soman-inhibited acetylcholinesterase in rat muscle but were moderately effective in the human and ineffective in the monkey muscle. PAM (Pre-Post) and PAM (Therapy) were ineffective in increasing soman-inhibited acetylcholinesterase activity in muscle from all species examined.

In the Pretreatment-Therapy situation there is a possibility that an oxime could shield the active site of acetylcholinesterase and thus protect it from phosphorylation by soman. HI-6, toxogonin and PAM have similar affinities for acetylcholinesterase as indicated by similar IC_{50} values (Clement 1981). SAD-128 is a more effective competitive inhibitor of acetylcholinesterase than the oximes evaluated, but it did not manage to protect the active site of acetylcholinesterase from inhibition by soman. These results suggest that the shielding of the active site of acetylcho-

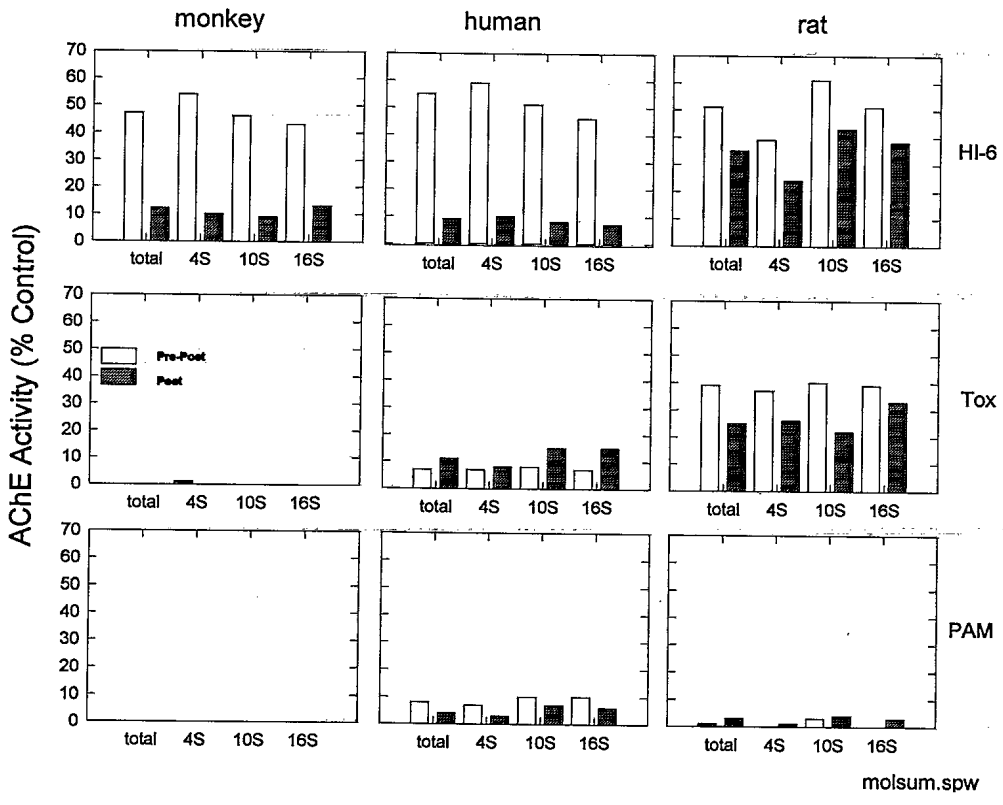

Fig. 1. Change in soman-inhibited acetylcholinesterase activity in rat, human and monkey striated muscle following various oximes and treatment regimens. The change in acetylcholinesterase activity of the "Pre-Post" group for each oxime is representative of the combined contribution of protection and reactivation of the oxime while the change in acetylcholinesterase activity of the "Post" group represents only reactivation activity of the oximes, since they were present only after exposure to soman. The acetylcholinesterase activities (% control) were calculated as the difference between the soman control group and the particular oxime treatment from the data presented in Tables 2, 3 and 4. The absence of a bar indicates that the value was zero

linesterase by the various oximes and SAD-128 was not a major factor in the results of the present investigation.

The level of acetylcholinesterase activity required in vivo for a beneficial therapeutic effect to be expressed is equivocal. Very small amounts of acetylcholinesterase activity, of the order of less than 10%, have been suggested to be of vital importance (Meeter and Wolthuis 1968; Wolthuis and Kepner 1978; Clement 1979; Heffron and Hobbiger 1979). Others have also reported that there is a narrow window of residual acetylcholinesterase in which the system functions normally before becoming chaotic (Koelle and Gilman 1946; Lomax et al. 1986; Clement 1991).

It is difficult to extrapolate the efficacy of HI-6 seen in animals to the human situation. In light of the results of this study combined with the pharmacokinetic data of HI-6 in primates (Clement et al. 1990) and man (Kusic et al. 1985; Clement et al. 1992, 1993), the monkey model may be an understimation of the efficacy of HI-6 in the human due to the fact that in the monkey the pharmacokinetics of HI-6 show a lower blood concentration and a faster elimination time. However, the fact that HI-6, in combination with atropine sulfate, is efficacious in primates against soman poisoning (Lipp and Dola 1980; Hamilton and Lundy 1989) should give us some confidence that this therapeutic regimen utilizing HI-6 would also be effective in humans.

SAD-128

As was found by Grubic and Tomazic (Grubic and Tomazic 1989), SAD-128 did improve the reactivation of soman-inhibited acetylcholinesterase produced by HI-6. It has been proposed that SAD-128 may be beneficial due to shielding of the acetylcholinesterase from inhibition by soman (Harris et al. 1978; Stalc and Sentjurc 1990), allosteric retardation of ageing of soman-inhibited acetylcholinesterase (Harris et al. 1978; Puu et al. 1986; Stalc and Sentjurc 1990), blockage of the nicotinic receptor ion channel (Tattersall 1993) or acceleration of oxime-induced reactivation (Harris et al. 1978). The results of the present study support the latter explanation. The protective effects of SAD-128 against membrane acetylcholinesterase activity may be related to the changes in the active site of the enzyme and/or can be moderated by the microviscosity changes of the membrane (Stalc and Sentjurc 1990).

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