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Pharmacokinetics and effects of HI 6 in blood and brain of soman-intoxicated rats: A microdialysis study

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Abstract

The bispyridinium oxime HI 6 (1-(((4-amino-carbonyl)pyridino)methoxy)methyl)-2-(hydroxyimino)methyl)-pyridinium dichloride monohydrate), combined with atropine, is effective for treating poisoning with organophosphate nerve agents. The protective action of HI 6 in soman poisoning has been attributed mainly to its peripheral reactivation of inhibited acetylcholinesterase. In the present study we investigated whether high intramuscular doses of HI 6 can reach the brain in a sufficient amount to reactivate inhibited brain acetylcholinesterase. Microdialysis probes were implanted in the jugular vein and striatum and dialysis samples were collected simultaneously from the two sites in awake, freely moving rats. Pharmacokinetic parameters of unbound HI 6 in blood and brain were calculated after administration of HI 6 (50, 75 or 100 mg/kg i.m.) in control rats and rats injected with soman (90 μg/kg s.c., 0.9 LD_{50}) 1 min before HI 6 treatment. We found that signs of soman poisoning correlated positively to acetylcholinesterase inhibition and negatively to the concentration of unbound HI 6 in the brain and that soman intoxication significantly decreased uptake of HI 6 into the brain. © 1997 Elsevier Science B.V.

Keywords: Dopamine; HI 6; Microdialysis; Pharmacokinetics; Acetylcholine esterase; Soman; (Rat)

1. Introduction

The bispyridinium oxime HI 6 (1-(((4-amino-carbonyl)pyridino)methoxy)methyl)-2-(hydroxyimino)-methyl)-pyridinium dichloride monohydrate), in combination with atropine, is today regarded as one of the most effective agents for treating intoxication with organophosphate inhibitors of acetylcholinesterase such as soman, sarin and Vx (Clement, 1981, 1982, 1983; Boskovic et al., 1984; Wolthuis et al., 1981a,b; Göransson-Nyberg et al., 1995). The main effects of HI 6 are generally considered to be due to the reactivation of inhibited acetylcholinesterase (Clement, 1981; Puu et al., 1986), but other important mechanisms may also be involved (Reithmann et al., 1988; Van Helden et al., 1991, 1994). Soman exerts its effects both on the central nervous system and in the periphery, whereas HI 6 seems to reactivate acetylcholinesterase mostly in the periphery (Clement, 1982; Lundy and Shih, 1983) a finding which has been assumed to explain the mechanism of the protective action of HI 6 in soman poisoning. The access of HI 6 to the central nervous system (CNS) is discussed and it has been demonstrated that HI 6 penetrates the blood–brain barrier, but not in a sufficient concentration to reactivate the brain acetylcholinesterase after soman intoxication (Cassel and Fosbraey, 1996).

Although HI 6 is rapidly eliminated from the blood stream, its access to and effect in the central compartment has been demonstrated (Ligtenstein and Kossen, 1983; Klimmek and Eyer, 1986; Ligtenstein et al., 1988; Lundy et al., 1990, Van Helden et al., 1994; Cassel and Fosbraey, 1996). In a study in mice, Clement (1992) found that after intoxication with sarin, HI 6 penetrated the blood–brain barrier in a sufficient concentration to have biochemical and physiological effects.

Exposure to high doses of soman results in a variety of signs of poisoning involving the cholinergic system. However, while central muscarinic mechanisms appear to be responsible for the initiation of soman-induced seizures, other neurotransmitter systems may be involved in the

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propagation or maintenance of the seizures (Shih et al., 1991). A correlation between an increase in the severity of signs of poisoning, with neurochemical changes and extracellular levels of dopamine has previously been demonstrated (Cassel and Fosbraey, 1996). Changes in the levels of noradrenaline, GABA and the metabolites of dopamine have been reported following soman intoxication in the guinea-pig (Fosbraey et al., 1990). However, Reithmann et al. (1988) observed an increase in the dopamine but not in the noradrenaline level after soman poisoning and noted that the increase in the dopamine level was absent after HI 6 treatment.

Brain HI 6 levels may play a critical role in the prevention of signs of poisoning following soman intoxication. Recently, it was found in soman-intoxicated animals that less severe clinical signs of poisoning resulted when more HI 6 penetrated into the brain, or perhaps, that more HI 6 penetrated when less severe signs were observed (Cassel and Fosbraey, 1996). Despite the fact that HI 6 produced significant reactivation of inhibited plasma acetylcholinesterase in soman-intoxicated animals, no evidence of enzyme reactivation in the brain tissue was observed. However, with higher doses of HI 6 than those used in the last-mentioned study, it may be possible to achieve penetration of HI 6 into the brain in a sufficient concentration to reactivate inhibited acetylcholinesterase.

The purpose of the present study was to determine the dose of HI 6 required to reactivate soman-inhibited brain acetylcholinesterase in the rat, and to find out whether there are any correlations between signs of poisoning, the levels of biogenic amines, the concentration of HI 6, and acetylcholinesterase. The technique of microdialysis has made it possible to follow the distribution of drugs simultaneously in the blood and brain and at the same time to measure the levels of biogenic amines in the extracellular fluid (Ungerstedt, 1984). In the present study, the technique of microdialysis was used to determine the striatal and blood levels of unbound HI 6 following peripheral administration of different doses of HI 6 (50, 75 or 100 mg/kg HI 6; 10 mg/kg atropine, i.m.) in conscious, freely-moving rats. The results were compared with those obtained in animals intoxicated with soman (90 μg/kg s.c.; 0.9 LD_{50}) 1 min before HI 6 administration. Simultaneously, the levels of dopamine, 3,4-dihyroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the striatal extracellular space, and the inhibition of acetylcholinesterase in the blood and different brain regions was determined.

2. Materials and methods

2.1. Animals

Male, Wistar rats (210–260 g) from Möllegaard, Denmark, were used throughout the study. The animals were acclimatised in the animal department for at least 1 week prior to the experiments. The room temperature was 21–24°C and humidity 50 ± 5%. Artificial light was the only source of light and the animals were set on a 12 h light/dark cycle with lights on at 6.00 a.m. All animals had access to food and water ad libitum before drug administration. All the animal experiments were approved by the Regional Research Ethical Committee in accordance with Swedish laws (SFS 1988:539, LSFS 1989:41).

2.2. Chemicals

The bispyridium oxime HI 6 (1-(((4-aminocarbonyl)pyridino)methoxy)methyl)-2-(hydroxyimino)methyl)-pyridinium dichloride monohydrate) was obtained from Astra, Sweden. Soman (pinacolyl methylphosphonofluoridate; > 95% pure) was synthesised at the Department of Chemistry, FOA NBC, Umeå, and diluted to its final concentration with sterile water on the day of the experiment.

2.3. Microdialysis guide implantation

The microdialysis procedure was carried out as described by Cassel et al. (1995), with the addition of a probe in the jugular vein for blood dialysis. The rats were rendered unconscious with the neuroleptanalgesic cocktail of Hypnorm® (Janssen, UK) and Dormicum® (Roche, Switzerland), mixed with water in the ratio 1:1:2 (Hypnorm/Dormicum/water) and injected (3.3 ml/kg i.p.) before surgery. A microdialysis guide (Carnegie Medicine, Sweden) was implanted in the striatum, using a stereotaxic frame (David Kopf), at the following co-ordinates relative to the bregma: R +1.2, L +2.0, V −3.3 and a microdialysis probe (CMA/20; membrane length 4 mm; outer diameter 0.5 mm) was inserted in the jugular vein. During surgery, the core temperature of the rat was kept at 37.5°C.

2.4. Postoperative period

After recovery from anaesthesia, the rats were housed separately on a 12 h light/dark cycle (light 6.00 a.m to 6.00 p.m) with free access to food and water. They were trained each day to become acclimatised to the equipment, which limited the stress on the day of the experiment resulting from exposure to a novel environment.

2.5. Experimental procedure

The experiment started 1–2 days after surgery. Under light anaesthesia with Efrane® (Abbott, Italy), the dummy probe in the brain was removed from the guide and the microdialysis probe (CMA/12; membrane length 3 mm; outer diameter 0.5 mm) was inserted and locked into position. A blood sample of 50 μl was taken from a tail vein in each rat, for estimation of the basal acetylcholinesterase activity. The rat was placed in a freely moving

equipment (CMA/120). To obtain dialysis equilibrium, the brain and blood microdialysis probes were perfused with a buffer solution (a mixture of 147 mM NaCl, 4 mM KCl, 1.5 mM $CaCl_2$ and 0.15 mM Hepes) for 60 min at a rate of 2 μl/min.

After this period, the recovery of the brain and blood probes was determined in each animal by the method of retrodialysis (Ståhle, 1991). By perfusing the probes with a known concentration of HI 6 (C_{in}), the recovery (%), expressed as $(C_{in} - C_{out}/C_{in}) \times 100$ could be obtained. The probes were then dialysed with blank perfusate for at least 2 h in order to enhance the washout of drug from the tissue. The experiment started with three basal fractions (20 min/fraction), after which soman (90 μg/kg s.c.; 0.9 LD_{50}) or saline was injected 1 min before i.m. injection of HI 6 with atropine (50, 75 or 100 mg/kg HI 6; 10 mg/kg atropine). Nine additional fractions were collected before the experiment was terminated.

Overt signs of poisoning (activity, behaviour, salivation, paddling, vocalising, sniffing, chewing, tremor, fasciculation and lachrymation) were noted on a checklist for motor activity and toxic signs during the experiment. The animals were classified into three groups. *No signs*: no clinical signs of poisoning; *mild signs*: chewing and increased activity and *severe signs*: mild signs and in addition head tics, marked hyperactivity, fasciculation, continuous tremor, convulsions, salivation and lachrymation.

At the end of the experiment, the rat was anaesthetised with enflurane and killed by decapitation. The probe was carefully removed and its location in the striatum assessed by visual examination of the brain section. For estimation of acetylcholinesterase activity, 50 μl of blood was collected as above and the contralateral half of the brain was dissected into six different parts (striatum, cerebellum, hippocampus, cortex, hypothalamus, medulla/pons).

2.6. Determination of biogenic amines and HI 6

The assays of dopamine, DOPAC, 5-HIAA, HVA and HI 6 were performed according to Cassel and Fosbraey (1996) with some modifications. The mobile phase was an aqueous solution containing 127 mM sodium dihydrogen phosphate, 0.85 mM EDTA, 17 ml PIC B8 (pair ion chromatography, octanesulphonic acid, Waters), 80 ml methanol and 860 ml H_2O, pH 4.0. The peak of interest was separated on a reverse phase C18 high performance liquid chromatography (HPLC) column (Chrompack ChromSpher 5 μm particle size, 2 × 10 cm cartridge). The HPLC system was equipped with an electrochemical detector (BAS LC-4C) set at a working potential of +0.75 V, for the detection of dopamine, DOPAC, HVA and 5-HIAA, in series with a UV detector (BAS/UV 116)) set at a wavelength of 295 nm for the detection of HI 6. For quantification, peak area ratios of dialysate samples were compared with external standards. The detection limit applied was a signal to noise ratio of 3.

2.7. Acetylcholinesterase measurement

The acetylcholinesterase activity was measured in the blood and the different brain regions of all animals receiving soman and/or HI 6, using a modified method of Augustinsson et al. (1978). In a separate control group, homogenates from different brain areas were analysed for basal acetylcholinesterase activity. The 50 μl blood sample was added to 950 μl 0.1% Triton and the brain parts (about 0.1 g) were homogenised at a concentration of 1/10 with 0.1 M sodium phosphate buffer, pH 8.0, mixed with 0.1% Triton x-100. The brain homogenate was diluted 10- to 20-fold and 25 μl of both brain and blood solutions was then mixed with 75 μl of PDS buffer (0.28 mM 4,4′-dithiodipyridine diluted in methanol) and incubated for 15 min on a shaker at room temperature. After incubation, the reaction was started by adding 100 μl 2 mM acetylthiocholine iodide buffer and the changes in absorbance were measured for 10 min on a Labsystems EMS Reader MF (wavelength 324 nm). A software program calculated the slope from the four points that had the highest influence on the slope.

2.8. Pharmacokinetic analysis

Pharmacokinetic parameters of HI 6, in blood and brain, were individually estimated for each animal by standard methods (Hladky, 1990). Unbound HI 6 concentrations versus time plots from the intramuscular route of administration were fitted to the one-compartment pharmacokinetic model with first-order absorption. The area under the curve (AUC) was calculated using the trapezoidal rule and was extrapolated to infinity with the terminal elimination rate constant (β), according to defined extrapolation methods. β was calculated by least-squares regression from the terminal linear part of the curves when semilogarithmic co-ordinates were used. Clearance (CL) was calculated from the equation CL = dose/AUC. The volume of distribution (V_D) was calculated as $V_D = Cl/\beta$. Also calculated from the data was the half-time of elimination of the terminal phase, $t_{1/2}\,\beta = \ln 2/\beta$. The maximum concentration (C_{max}) and the time at which the maximum occurred (t_{max}) were determined from the individual concentration–time profiles.

2.9. Statistical analysis

Principal component analysis (PCA) is a projection method that combines the included variables down to a few underlying descriptive dimensions and summarises the systematic variation in a large data table (Wold et al., 1984). PCA is primarily used to get an overview of dominant patterns or major trends in a data matrix. In biomedical research the use of this method has been demonstrated by Ståhle and Wold (1988) and Cassel and Fosbraey (1996).

Whereas PCA can reveal the structure in a set of data, projection to latent structures (PLS) can be used to disclose the structure in the data on the basis of external information (Dunn et al., 1984; Ståhle and Wold, 1988). In PLS, variables such as symptoms and treatment can be included in the model, which gives 2 plots, a loading and a score plot. The loading plot shows the correlation between the response variable Y (here, signs) and the descriptor matrix (here, the variables), while the score plot gives information concerning the relationships between objects. The number of significant terms of the PLS model is determined by cross-validation to ensure that the components are significant (Wold, 1978). All calculations were performed with the Simca-S software package (Umetri S-901 24 Umeå, Sweden).

The pharmacokinetics of HI 6, acetylcholinesterase activity and biogenic amines were initially compared among groups, using ANOVA. The Bonferroni method was used for parametric comparisons. When a probability of $P < 0.05$ was found, comparisons were made between groups using an unpaired Student's t-test and within groups by the paired t-test with $P < 0.05$ as significant. Data are presented as mean values ± S.E.M.

3. Results

3.1. Pharmacokinetics of HI 6

The mean concentrations of unbound HI 6 in the blood and brain after administration of different doses of the oxime in controls and in soman-intoxicated rats are shown in Fig. 1. The mean pharmacokinetic variables are summarized in Tables 1 and 2. The area under the HI 6 concentration versus time curve (AUC, Table 1) did not differ significantly between the control and soman groups treated with 50 or 75 mg/kg of HI 6. In animals treated with 100 mg/kg of HI 6, however, AUC for HI 6 in the blood was significantly higher in the group intoxicated with soman than in the control group ($P < 0.01$). AUC for HI 6 in the brain was compared with AUC for HI 6 in the blood in each animal and the results are presented as %AUC brain/blood in Table 1. It is seen that 17.7 ± 2.2 of HI 6 reached the brain under control conditions and 9.0 ± 1.5 during soman intoxication; this difference was significant ($P < 0.05$).

The elimination half time ($t_{1/2}\,\beta$, Table 1) for HI 6 did not differ significantly between the groups of animals, but

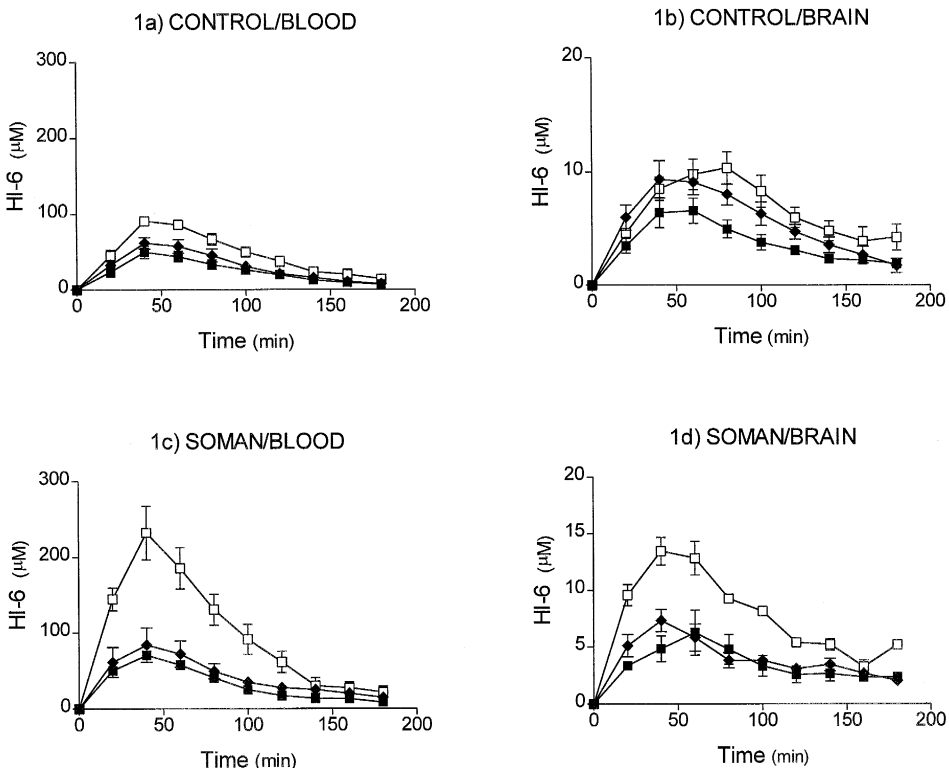

Fig. 1. Time–concentration curves of the unbound oxime HI 6 in the blood and brain after administration of HI 6 in different doses (■, 50; ♦, 75 and □, 100 mg/kg i.m.) in control rats and in rats intoxicated with soman (90 μg/kg s.c.). (a and b) Concentration of HI 6 in the blood and brain of control animals, respectively. (c and d) Concentration in the blood and brain of soman-intoxicated animals respectively. Each point represents the mean ± S.E.M. For n and P-values, see Table 1Table 2.

Table 1
Pharmacokinetic parameters of HI 6 in the blood and brain after i.m. administration of HI 6 in controls and in rats intoxicated with soman

	Treatment HI 6 i.m. (mg/kg) (*n*)	$t_{1/2}\,\beta$ (min)		AUC (μg/ml per min)		% AUC	V_D (l/kg)		Cl_{tot} (ml/min per kg)	
		blood	brain	blood	brain	brain/blood [b]	blood	brain	blood	brain
Control saline s.c.	50 (5)	20.5 ± 2.4	33.2 ± 9.4	1677.7 ± 288.1	231.7 ± 40.6	14.9 ± 2.7	0.97 ± 0.16	11.8 ± 4.0	34.3 ± 7.0	243.4 ± 42.0
	75 (4)	18.0 ± 2.0	23.6 ± 3.6	2113.0 ± 343.0	440.2 ± 28.9	23.5 ± 4.1	1.00 ± 0.16	6.0 ± 1.3	40.8 ± 8.4	173.4 ± 11.7
	100 (5)	20.9 ± 3.7	35.2 ± 6.7	3227.8 ± 285.8 [a]	436.6 ± 54.1	14.1 ± 0.3	0.93 ± 0.11	12.5 ± 3.0	32.0 ± 2.8 [a]	248.5 ± 40.4
Soman, 90 μg/kg s.c.	50 (5)	19.4 ± 2.3	34.0 ± 8.8	2192.8 ± 144.1	213.9 ± 43.6	9.9 ± 2.4	0.66 ± 0.11	13.0 ± 3.9	23.2 ± 1.5	297.3 ± 84.2
	75 (5)	29.5 ± 7.2	32.9 ± 2.7	2867.8 ± 580.7	239.2 ± 45.3	8.7 ± 1.4	1.40 ± 0.45	18.5 ± 4.9	30.5 ± 5.6	369.0 ± 75.9
	100 (5)	16.8 ± 2.9	27.1 ± 4.3	6886.8 ± 960.4 [a]	503.5 ± 27.2	8.4 ± 1.8	0.39 ± 0.10	7.8 ± 1.1	15.8 ± 2.3 [a]	200.8 ± 10.8

$t_{1/2}\,\beta$, terminal half-time of elimination; AUC, area under the free concentration–time curve extrapolated to infinite time.
V_D, volume of distribution; Cl_{tot}, total plasma clearance. All results are expressed as mean ± SEM.
Number of animals in parentheses.
[a] $P < 0.01$ for comparison between control and soman groups treated with 100 mg/kg HI 6.
[b] $P < 0.01$ for comparison between all controls and all soman-intoxicated rats.

Table 2
Pharmacokinetic parameters of HI 6 in the blood and brain after i.m. administration of HI 6 in controls and in rats intoxicated with soman

	Treatment HI 6 i.m. (mg/kg) (n)	C_{max} ($\mu g \cdot ml^{-1}$)		t_{max} (min)	
		blood	brain	blood	brain
Control saline s.c.	50 (5)	18.8 ± 3.1	2.7 ± 0.4	42.9 ± 2.2 [a]	56.1 ± 5.2 [a]
	75 (4)	23.4 ± 3.9	3.9 ± 0.7	44.0 ± 4.1 [b]	60.0 ± 0.0 [b]
	100 (5)	35.1 ± 1.9 [c]	4.2 ± 0.5	44.0 ± 4.1 [b]	68.0 ± 4.9 [b]
Soman, 90 μg/kg s.c.	50 (5)	26.6 ± 1.8	2.5 ± 0.7	35.0 ± 6.6 [a]	60.0 ± 0.0 [a]
	75 (5)	31.7 ± 8.5	2.8 ± 0.4	40.0 ± 0.0 [a]	53.3 ± 6.7 [a]
	100 (5)	87.6 ± 13.4 [c]	5.1 ± 0.5	40.0 ± 0.0 [a]	53.3 ± 6.7 [a]

C_{max}, maximal concentration; t_{max}, time taken to reach the observed maximum.
All results are expressed as mean ± S.E.M. Number of animals in parentheses.
[a] $P < 0.05$ for comparison between blood and brain.
[b] $P < 0.01$ for comparison between blood and brain.
[c] $P < 0.01$ for comparison between control and soman groups treated with 100 mg/kg HI 6.

was found to be significantly shorter in the blood than in the brain when all animals were compared irrespective of treatment ($P < 0.02$).

The time taken to reach the maximal HI 6 concentration in the blood and in brain was calculated in each rat. The means of these times (t_{max}) are shown in Table 2. The time taken to reach the peak concentration did not differ significantly between the different groups, but in each case the maximum concentration was reached several minutes later (between 10–30 min) in the brain than in the blood (for P-values see Table 2). Furthermore, the concentration in the brain was about 10 times lower than in the blood. The peak concentration of HI 6 (C_{max}, Table 2) did not differ significantly between the control and soman groups treated with 50 or 75 mg/kg of HI 6. Among the animals treated with 100 mg/kg of HI 6, however, C_{max} in the blood was significantly higher in the group intoxicated with soman than in the control group ($P < 0.01$).

3.2. Acetylcholinesterase activity

The mean inhibition of acetylcholinesterase activity in the blood and in the different brain regions in animals given soman alone and soman followed by different doses of HI 6 is shown in Table 3. Soman per se (90 μg/kg s.c., 0.9 LD_{50}) significantly inhibited the acetylcholinesterase activity in the blood by a mean of 91.8 ± 0.1% and in the brain by 76.9 ± 2.6%. Intramuscular administration of the lower dose of HI 6 (50 mg/kg) had no effect on the acetylcholinesterase activity in blood or in brain. When the intoxicated rats were treated with HI 6 in doses of 75 and 100 mg/kg, the acetylcholinesterase activity increased significantly in the blood by 23.4 ± 2.1 and 57.2 ± 4.2, respectively ($P < 0.05$), and in the cerebellum by 24.8 ± 3.9 and 51.9 ± 8.8, respectively ($P < 0.05$), indicating a reactivation of the enzyme. With the highest dose of HI 6, 100 mg/kg, a significant reactivation was also observed in hypothalamus, by 28.3 ± 4.5 ($P < 0.05$).

3.3. Signs of poisoning

Signs of poisoning (no signs, mild signs and severe signs), treatment, the concentration of biogenic amines and HI 6 at time points between 20 and 180 min were used as variables in a PLS analysis. Before analysis, the data were scaled to unit variance, in order to minimize statistical bias

Table 3
The inhibition of acetylcholinesterase activity in soman-intoxicated animals after treatment with different doses of HI 6 (0, 50, 75, or 100 mg/kg)

	Acetylcholinesterase inhibition, % relative to controls [b]			
	soman, 90 μg/kg s.c. (n)	soman + HI 6 50 mg/kg i.m. (n)	soman + HI6 75 mg/kg i.m. (n)	soman + HI 6 100 mg/kg i.m. (n)
Blood	91.8 ± 0.1 (3)	79.0 ± 5.9 (6)	71.2 ± 2.7 [a] (6)	40.4 ± 6.6 [a] (6)
Striatum	81.0 ± 1.8 (3)	52.3 ± 9.8 (6)	49.4 ± 4.3 (6)	50.4 ± 9.1 (6)
Hippocampus	76.0 ± 12.6 (3)	84.1 ± 1.0 (6)	78.4 ± 4.6 (6)	54.1 ± 8.9 (6)
Cerebellum	83.3 ± 0.1 (3)	66.8 ± 3.8 (6)	51.7 ± 5.7 [a] (6)	40.2 ± 11.9 [a] (6)
Hypothalamus	81.0 ± 3.9 (3)	69.9 ± 7.9 (6)	52.9 ± 6.7 (6)	59.2 ± 5.9 [a] (6)
Cortex	75.1 ± 6.9 (3)	82.0 ± 2.1 (6)	67.6 ± 15.5 (6)	63.8 ± 12.9 (6)
Medulla/pons	64.7 ± 3.3 (3)	52.7 ± 11.0 (6)	64.8 ± 4.1 (6)	58.3 ± 7.8 (6)

All results are expressed as mean ± S.E.M. Numbers of animals in parentheses.
[a] $P < 0.05$ for soman-intoxicated animals treated with HI 6 compared with untreated soman-intoxicated rats.
[b] Controls = 0% inhibited.

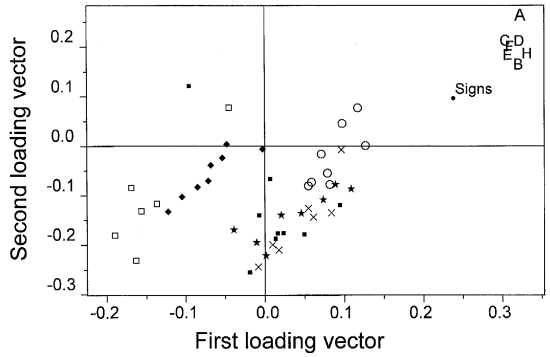

Fig. 2. This figure shows the plot of the second loading vector versus the first one for all variables in the PLS analysis. The variables are indicated as: ◆, unbound HI 6 levels in the striatal extracellular fluid (ECF); ○, unbound HI 6 levels in the blood; ■, HVA levels in the striatal ECF; ★, dopac levels in the striatal ECF; □, dopamine levels in the striatal ECF; ×, 5-HIAA levels in the striatal ECF. The acetylcholinesterase inhibition is indicated for: (A) blood; (B) cortex; (C) cerebellum; (D) striatum; (E) medulla and (F) hippocampus. The loading plot shows that signs of poisoning correlate positively to acetylcholinesterase inhibition and negatively to the concentration of unbound HI 6 in the brain.

due to differences in chemical concentrations. The PLS on the resulting 58 variables (symptoms, acetylcholinesterase inhibition, dopamine, DOPAC, HVA, 5-HIAA and HI 6) gave a significant two-compartment model which in total described 79% (67.6 + 11.5%) of the total variance of the data set. The responding cross-validated result was 61.1%. In the loading plot (Fig. 2), variables which are close to signs and far from the zero point show a positive correlation, while those in opposite areas of the plot are negatively correlated. The variables near to the zero point have no effect of the signs of poisoning. The loading plot thus shows that signs of poisoning correlated positively to acetylcholinesterase inhibition and negatively to the concentration of unbound HI 6 in the brain (Fig. 2). The concentration of unbound HI 6 in the blood did not correlate with the signs of poisoning observed.

Furthermore, there was a significant increase in the dopamine concentration in the brain in untreated soman-intoxicated rats ($P < 0.05$, Fig. 3). This increase was not observed in soman-intoxicated animals treated with either 50, 75 or 100 mg/kg of HI 6. However, among the soman-intoxicated animals treated with 50 mg/kg of HI 6, there were some with severe symptoms that showed an increase in the dopamine level and others with mild symptoms that did not show such an increase. A significant value was not obtained in this group.

The DOPAC, HVA and 5-HIAA concentrations in the striatum did not differ significantly between the control rats, rats intoxicated with soman without further treatment and intoxicated rats treated with different doses of HI 6.

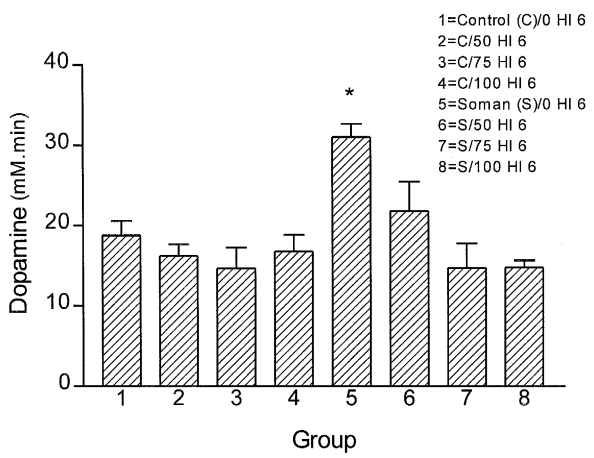

Fig. 3. Dopamine levels in the rat brain after administration of HI 6 in different doses (0, 50, 75, and 100 mg/kg i.m.) in control rats and in rats intoxicated with soman (90 μg/kg s.c.). Mean ± S.E.M. The dopamine level in the group given soman alone differed significantly from that in all groups ($^{*}P < 0.05$) with the exception of group 6.

4. Discussion

The results of the present study demonstrate the penetration of intramuscularly administered HI 6 into the brain and the effectiveness of a combination of HI 6 and atropine in reversing the toxic effects of soman in awake, freely moving rats. The findings also show that when soman-intoxicated rats are treated with higher doses of HI 6 (75 or 100 mg/kg), the acetylcholinesterase activity is less inhibited in some brain regions, indicating reactivation of the enzyme.

Several in vivo experimental methods have been used to study blood–brain barrier transport (Fenstermacher et al., 1981). In general they can be divided into single and multiple passage techniques, whereby mostly a comparison is made between blood and tissue drug concentrations at a single point in time, following systemic administration. A limitation of these methods is that only a single concentration point is obtained within each individual animal. By the technique of in vivo microdialysis (Ungerstedt, 1984) it is possible to monitor the blood and tissue of animals continuously for metabolic and pharmacokinetic purposes (Ståhle, 1991; Ekblom et al., 1992). As there is no net fluid exchange, continuous sampling for long periods of time without disturbing the pharmacokinetics is possible. Furthermore, it has been shown in several studies that the blood–brain barrier remains intact shortly after insertion of the microdialysis probe (De Lange et al., 1994).

In the present study we have demonstrated, by the use of the technique of in vivo microdialysis in the striatum, that intramuscularly administered HI 6 at different doses passes the blood–brain barrier in both control rats and rats intoxicated with soman (Fig. 1). The rapid phase of the HI 6 increase in the brain dialysate occurred within 30 min of injection and coincided with the absorption phase of HI 6 in the blood; the maximum of HI 6 in the brain dialysate, however, being 10 times lower than the maximum in the blood. Our results show that the uptake of HI 6 into the

brain is reduced during soman intoxication irrespective of the dose administered. Approximately 18% of the HI 6 reached the brain under control conditions and 9% during soman intoxication. This is in good accordance with findings by Cassel and Fosbraey (1996). The lower uptake of HI 6 into the brain during soman intoxication indicates an effect of a soman-induced vasoconstriction of the capillaries in the blood–brain barrier or a depressing effect on blood pressure. These results are conflicting and need to be further investigated, since it has been reported by others (Maxwell et al., 1987; Goldman et al., 1993) that soman increases the cerebral blood flow.

The significance of the effects of soman intoxication on the pharmacokinetics of HI 6 should not be ignored. Kepner and Wolthuis (1978) have reported that plasma levels of oxime are elevated in soman-intoxicated rats. The observation that soman reduces the blood flow in the kidney (Maxwell et al., 1987) may explain this effect of soman, since oxime elimination occurs primarily by renal excretion of unchanged drug (Simons and Briggs, 1983; Ligtenstein and Kossen, 1983; Kusic et al., 1985), for which the blood flow is the rate-limiting factor. In the present study, soman intoxication only interfered with the pharmacokinetics of HI 6 when the highest dose (100 mg/kg, Fig. 1c) was administered. In this group there was a significant increase in AUC and a higher C_{max} of HI 6 in the blood than in the control group treated with the same dose. These results can be explained by a combination of a reduced renal blood flow and the observation of Kusic et al. (1985) that HI 6 is not only filtered at the glomerulus but also actively secreted by the renal tubule cells. This active process may be saturated at higher doses of HI 6, leading to an increase in the concentration of HI 6 in the blood.

The beneficial effects of oximes are generally thought to be due to their ability to reactivate inhibited enzyme, although the protective effect have been attributed at least partly to actions other than enzyme reactivation. The role played by the oximes in the CNS is somewhat equivocal, however, since most of the very useful oximes are quaternary in structure. Although these oximes appear to cross the blood–brain barrier, at least in small amounts (Clement, 1982; Reithmann et al., 1988; Van Helden et al., 1994; Cassel and Fosbraey, 1996), the degree of reactivation of the brain acetylcholinesterase is not high. In some studies attempts have been made to enhance CNS effects of oximes by injecting them directly into the brain, with conflicting results (Lundy and Shih, 1983). In any case, it has previously been found difficult or impossible to correlate protective effects of the oximes with their ability to reactivate whole brain acetylcholinesterase (Van Helden et al., 1991, 1992, 1994, 1996).

In the present study intramuscular administration of the lower dose of HI 6 (50 mg/kg) apparently had no effect on the acetylcholinesterase activity in the blood or brain in soman-intoxicated rats. But when the intoxicated rats were treated with higher doses of HI 6, namely 75 and 100 mg/kg, there was a significant increase in acetylcholinesterase activity in the blood by approximately 23 and 57%, respectively, and in the cerebellum by approximately 25 and 52%, respectively, indicating reactivation of the enzyme. With the highest dose of HI 6, 100 mg/kg, significant reactivation was also observed in the hypothalamus, by approximately 28%. The relevance of our results for humans in case of soman poisoning has to be further evaluated, since the reactivation of soman inhibited human acetylcholinesterase is much more difficult to achieve than that in rats (De Jong and Wolring, 1983). However, if the oxime therapy is inducted on a very early stage of poisoning as in this study, a reactivation might be possible.

A marked reactivating effect on brain acetylcholinesterase was found in earlier studies when sarin-poisoned rats were treated with high doses of HI 6 (Clement, 1982; Lundy and Shih, 1983). Reithmann et al. (1988) observed in mice that a low dose of HI 6 (55 mg/kg) caused no reactivation of cerebral ChE, but had a marked effect in the serum. In contrast to the findings of Reithmann et al. (1988) and Klimmek and Eyer (1986), Clement (1982) reported some central reactivation by HI 6 in rats after low doses of soman, but used higher doses of HI 6 (125 mg/kg). As high doses of the oxime are necessary for these effects, other central effects than reactivation seem to be even less probable.

Earlier reports have shown that soman-induced convulsions are not related to enhanced cholinergic activity or muscarinic effects and that HI 6 does not protect against convulsions (Lundy and Shaw, 1983). But in the present study PLS showed that signs of poisoning correlated positively to acetylcholinesterase inhibition and negatively to the concentration of unbound HI 6 in the brain (Fig. 2). Soman-intoxicated animals with severe symptoms (convulsions) after treatment with HI 6 (irrespective of dose), show a higher degree of acetylcholinesterase inhibition and a lower concentration of HI 6 in the brain dialysate. In contrast, soman-intoxicated animals with mild symptoms after HI 6 treatment showed a lower degree of acetylcholinesterase inhibition and a higher concentration of HI 6 in the brain, confirming our earlier report (Cassel and Fosbraey, 1996). The concentration of unbound HI 6 in the blood did not correlate with the signs of poisoning observed in this study.

The severe signs of poisoning could also be due to higher levels of striatal dopamine in these animals, since a large increase in striatal dopamine with a wide variation in the magnitude of the response has been observed after electroconvulsive shock (Nomikos et al., 1991). Reithmann et al. (1988) noted an increase in the brain dopamine level in mice poisoned with soman. This increase was fully antagonised by atropine as well as by a combination of atropine and HI 6. It was shown in their experiments for the first time that not only atropine but also HI 6 alone almost completely prevented the increase in brain dopamine

level caused by soman. This is in good accordance with our finding of a significant increase in brain dopamine in untreated soman-intoxicated rats (Fig. 3). This increase was not observed in soman-intoxicated animals treated with 75 or 100 mg/kg of HI 6. However, among soman-intoxicated animals treated with 50 mg/kg of HI 6 there were some with severe symptoms that showed an increase in the dopamine level and others with mild symptoms that did not. The dopamine level showed no significant change in this group.

In conclusion, the principal results in this study are that HI 6 in higher doses penetrate into the brain in a sufficient concentration to reactivate inhibited acetylcholinesterase. Signs of soman poisoning correlate positively to the acetylcholinesterase inhibition and negatively to the concentration of unbound HI 6 in the brain. Furthermore, our results show a reduced uptake of HI 6 into the brain during soman intoxication irrespective of the dose administered.

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