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ORGAN TOXICITY AND MECHANISMS

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Therapeutic efficacy of the adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) against organophosphate intoxication

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Abstract The objective of the present study was to investigate whether reduction of central acetylcholine (ACh) accumulation by adenosine receptor agonists could serve as a generic treatment against organophosphate (OP) poisoning. The OPs studied were tabun (*O*-ethyl-*N*-dimethylphosphoramidocyanidate), sarin (isopropylmethylphosphonofluoridate), VX (*O*-ethyl-*S*-2-diisopropylaminoethylmethylphosphonothiolate) and parathion (*O*,*O*-diethyl-*O*-(4-nitrophenyl)phosphorothioate). The efficacy of the adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) against an OP intoxication was examined on the basis of the occurrence of clinical symptoms that are directly associated with such intoxication. CPA (1–2 mg/kg) effectively attenuated the cholinergic symptoms and prevented mortality in lethally tabun- or sarin-intoxicated rats. In contrast, CPA (2 mg/kg) proved to be ineffective against VX or parathion intoxication. Intracerebral microdialysis studies revealed that survival of sarin-poisoned and CPA-treated animals coincided with a minor elevation of extracellular ACh concentrations in the brain relative to the baseline value, whereas an 11-fold increase in transmitter levels was observed in animals not treated with CPA. In VX-intoxicated rats, however, the ACh amounts increased 18-fold, irrespective of treatment with CPA. The striatal acetylcholinesterase (AChE) activity following a lethal sarin intoxication was completely abolished in the vehicle-treated animals, whereas 10% and 60% AChE activity remained in animals treated with 2 mg/kg CPA 1 min after or 2 min prior to the poisoning, respectively. In VX-intoxicated animals the AChE activity in the brain was strongly reduced (striatum 10%, hippocampus 1%) regardless of the CPA treatment. These results demonstrate that CPA is highly effective against tabun or sarin poisoning, but fails to protect against VX or parathion. Survival and attenuation of clinical signs in tabun- or sarin-poisoned animals are associated with a reduction of ACh accumulation and with protection of AChE activity in the brain.

Keywords Organophosphate · N^6-Cyclopentyladenosine · Acetylcholine release · Adenosine A_1 receptor

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Introduction

Organophosphates (OPs) are highly toxic compounds that irreversibly inhibit the acetylcholinesterase (AChE)-mediated metabolism of acetylcholine (ACh), which subsequently results in excessive and toxic amounts of extracellular ACh in the cholinergic synapses. Exposure to an OP requires immediate medical intervention, which is based on skin decontamination, AChE reactivation, cholinergic antagonism and suppression of seizure activity to prevent neurotoxicity (Holstege et al. 1997). However, in many instances this treatment appears to be inadequate. In nerve gas-poisoned primates it has been shown that the current treatment could not prevent neuronal brain damage and incapacitation (Hayward et al. 1990; Lallement et al. 1998; Shih and McDonough 1997; van Helden et al. 1996). Moreover, the oximes that are used to reactivate the inhibited AChE did not appear to be equally effective against all nerve agents, i.e. the treatment lacks generic applicability (Dawson 1994; van Helden et al. 1996; Worek et al. 1998).

Therefore, new strategies need to be developed in order to improve the current treatment of OP poisoning. In this respect a possible role for the adenosine A_1 receptor-mediated inhibition of ACh was explored recently (van Helden and Bueters 1999; van Helden et al. 1998). This approach might restore the imbalance between synaptic excess of ACh and reduced activity of AChE in case of OP poisoning, thereby preventing ACh accumulation and the subsequent emergence of brain pathology. Recently, we characterized the inhibitory effects on ACh release of a series of adenosine A_1 receptor agonists in an in vitro synaptosomal preparation and in situ by using intracerebral microdialysis (Bueters et al. 2000). Moreover, very promising results were obtained in soman-intoxicated rats treated with the adenosine A_1 receptor agonist N^6-cyclopentyladenosine (CPA) and the non-selective adenosine agonist 5'-*N*-ethylcarboxamidoadenosine (NECA) (van Helden et al. 1998). These authors demonstrated that CPA and NECA, administered 1 min after soman poisoning, prevented the main cholinergic symptoms and mortality.

The objective of this study was to further investigate the concept that the CPA-mediated reduction in accumulation of ACh could serve as a generic treatment in the case of tabun (*O*-ethyl-*N*-dimethylphosphoramidocyanidate), sarin (isopropylmethylphosphonofluoridate), VX (*O*-ethyl-*S*-2-diisopropylaminoethyl-methylphosphonothiolate) and parathion (*O*,*O*-diethyl-*O*-[4-nitrophenyl]phosphorothioate) intoxication.

For that purpose groups of rats were intoxicated with a lethal dose of the above-mentioned agents, followed by CPA treament 1 min later. Possible cholinergic symptoms were monitored and survival time recorded. A second group of animals was equipped with striatal microdialysis probes to measure the corresponding ACh concentrations in the brain. Animals were intoxicated with sarin or VX and treated with CPA, and the changes in ACh were monitored on-line. At the end of the experiment, the brain tissue was assayed for residual AChE activity.

Materials and methods

Animals

Male Wistar rats (270–380 g; Harlan BV, Horst, The Netherlands) were housed at two or three animals per cage and allowed to become accustomed to standard conditions for at least 1 week. Temperature was kept at 19–22°C, relative humidity at 55–65% and a 12-h light/12-h dark cycle was maintained (lights on at 700 hours). Acidified water and standard rodent chow (Teklad Global Diet; Harlan BV) were freely accessible. The Ethical Committee on Animal Experimentation of TNO approved all experiments described.

Chemicals

N^6-Cyclopentyladenosine was purchased from Research Biochemicals Inc. (Zwijndrecht, The Netherlands). Acetylcholinesterase (AChE), choline oxidase, triton-X, neostigmine bromide and acetylcholine (ACh) were obtained from Sigma Chemical Co. (Zwijndrecht, The Netherlands). Kathon CG (1.5% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one) was provided by Rohm and Haas (Croyden, UK). LiChrosorb NH_2 was purchased from E. Merck (Amsterdam, The Netherlands). Buffered saline was obtained from NPBI BV (Emmer-Compascuum, The Netherlands). Hypnorm was purchased from Janssen Pharmaceutica (Beerse, Belgium) and Dormicum was delivered by Roche Nederland BV (Mijdrecht, The Netherlands). [^{3}H]-Acetylcholine iodide (37.0 MBq/5 ml ethanol) was obtained from NEN Research Products (Boston, Mass., USA). Our Chemistry Department provided sarin, tabun, VX and parathion. The other chemicals used, were of standard purity and purchased from renowned companies. For high performance liquid chromatography (HPLC) analysis the highest purity grade was used.

All solutions were prepared with water tapped from a Milli-Q system (Millipore SA, Molsheim, France). CPA was dissolved in a mixture of 10% ethanol and 90% saline; parathion was dissolved in arachidon oil; tabun, sarin and VX were dissolved in isopropyl alcohol and further diluted in saline directly before use.

Clinical signs

Rats ($n = 5$–8) were injected according to the following regimen:

1. Tabun 386 μg/kg s.c., followed by CPA 0, 1 or 2 mg/kg i.m. after 1 min
2. Sarin 144 μg/kg s.c., followed by CPA 0, 1 or 2 mg/kg i.m. after 1 min
3. VX 24 μg/kg s.c., followed by CPA 0 or 2 mg/kg i.m. after 1 min
4. Parathion 12 mg/kg s.c. followed by CPA 0 or 2 mg/kg i.m. after 1 min

Doses of the various OPs were obtained from Ballantyne and Marrs (1992). The intoxicated rats were monitored and the relevant clinical symptoms were registered. During the first hour after intoxication the condition of the rat was scored every 2 min, and then at 15 min intervals for the next 5–6 h. Final assessments were made after 24 h. The following symptoms were scored:

1. Chewing: a clear chewing-like movement of the rat in which the entire head is involved as a consequence of increasing saliva production.
2. Salivation: extensive drooling.
3. Convulsions: involuntary tensed movement in which the entire body is involved. The rat looks mentally dissociated from the environment and is refractory to stimulatory impulses.
4. Respiratory distress: low respiratory rate and heavy breathing, often accompanied with some rattling, directing at obstruction in the throat.

Surgical procedure

Rats were anaesthetized with 2.7 ml/kg FFM-mix (1.25 mg/ml midazolam, 2.5 mg/ml fluanisone, 0.079 mg/ml fentanyl citrate) via a single intraperitoneal injection. A concentric microdialysis probe was stereotactically (KOPF Instruments, Tujunga, Calif., USA) implanted in the caudate putamen (A 0.5, L 3.0, V 6.8 mm relative to bregma and the dura mater) and was fixed with dental cement. A heating pad was used to maintain body temperature. The microdialysis probes used were self-constructed and made of a polyacrylonitryl/sodium methyl sulfonate copolymer dialysis membrane (Filtral 12; Hospal BV, Breda, The Netherlands), of which 3.5 mm was exposed.

Microdialysis procedure

Following surgery, the rats were placed individually in a perspex cages (25×25×40 cm) with free access to food and water. They were allowed to recover for 14–38 h after the surgical procedure. At the beginning and end of each microdialysis experiment a 10 nM ACh standard was injected in duplicate, and, if necessary, corrections for

reduced sensitivity were made. The microdialysis probe was perfused with a Ringer solution at 2.0 μl/min delivered by a microinjection pump (CMA 100; CMA Microdialysis AB, Solna, Sweden). To obtain detectable quantities of ACh in the dialysate, 100 nM neostigmine was added to the perfusate. Each rat was connected directly to the injection valve, allowing on-line analysis of the microdialysates. The injection valve was automatically activated every 5 min. After a stabilization period during which basal ACh outflow was established (four samples within 10% variation), rats were intoxicated with 144 μg/kg sarin (s.c.) followed by 2 mg/kg CPA (i.m.) after 1 min or pretreated with 2 mg/kg CPA (i.m.), 2 min prior to the intoxication. Furthermore rats were intoxicated with 24 μg/kg VX (s.c.) with or without pretreatment with 2 mg/kg CPA (i.m.), 2 min prior to the intoxication.

HPLC instrumentation and ACh analysis

Microdialysate samples were assayed for ACh using an HPLC system previously described by Damsma et al. (1987) with some modifications. The system consisted of a LC-10AD VP pump (Shimadzu, Den Bosch, The Netherlands), a pulse damper (SSI; Alltech, Breda, The Netherlands), a refillable guard column (silica pellicular packing material; Alltech), an EC 125/2 Nucleosil 100-5 C18 AB analytical column (Aurora Borealis Control, Schoonebeek, The Netherlands), preloaded with 0.5% sodium lauryl sulfate, an enzyme reactor, an electrically actuated injector (VALCO VICI AG, Schenkon, Switzerland), an INTRO potentiostate equipped with a VT03 flow cell with a platinum work electrode (Antec Leyden BV, Hazerswoude, The Netherlands) and a chromatography data acquisition system (Chromeleon; Gynkotek, Germering, Germany). The post-column enzyme reactor contained immobilized AChE (80 U) and choline oxidase (40 U), which converted ACh to hydrogen peroxide, which was detected at +450 mV. The mobile phase consisted of a 166 mM potassium phosphate buffer (pH 8.5) containing 1 mM tetramethylammonium chloride, 0.79 mM ethylenediaminetetraacetic acid (EDTA) and 250 μl/l Kathon CG. The system was run at 0.35 ml/min and temperature was maintained at 30°C. Limit of quantification was 1 pmol/ml. Linear calibration curves were obtained in the range 5–1000 pmol/ml ($r > 0.990$). The intra-assay coefficients of variation for 10 and 100 pmol/ml were 2.9% and 4.1%, respectively. Inter-assay variability could not be determined due to variable enzyme activities in the post-column reactor between days.

AChE determination

At the end of the microdialysis experiment, each rat was killed and the brain was removed. The right hemisphere was stored in 4% formaldehyde to assess the location of the probe. From the left hemisphere, the striatal and the hippocampal (VX alone) tissues were dissected out and homogenized (900 rpm, 10% g/v homogenate) in ice-cold TENT buffer, which consists of 50 mM Tris, 1 M NaCl, 5 mM EDTA and 1% v/v Triton-X, pH 7.4. The homogenate was centrifuged at 1500 *g* for 10 min at 4°C (Beckman GS-6R; Beckman Instruments Inc., Palo Alto, Calif., USA). The supernatant was transferred in a clean tube, directly frozen in liquid nitrogen and stored at –70°C until analysis. After appropriate dilution, samples were assayed for AChE activity using a radiometric method previously described by van Helden et al. (1992).

Data presentation and statistical analysis

All results are given as the mean ±SEM. Statistical analysis of the data was performed using one-way ANOVA followed by the Student-Newman-Keuls post hoc test whenever appropriate. Differences were considered significant for $P < 0.05$.

Results

Therapeutic efficacy

Observed clinical signs in rats upon intoxication with tabun, sarin, VX or parathion, respectively, are shown in Fig. 1. For comparison, the previously reported results with soman-intoxicated rats (van Helden et al. 1998) are included (Fig. 1A).

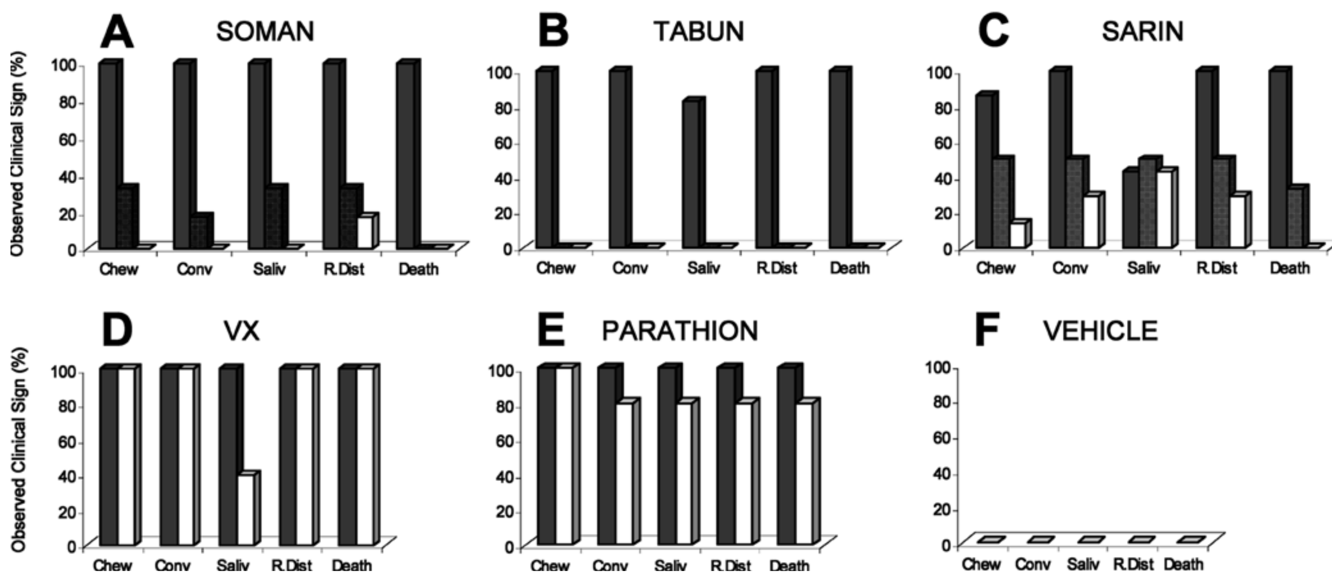

Fig. 1A–F. The therapeutic efficacy of N^6-cyclopentyladenosine (CPA) against the lethal intoxications (s.c.) with: **A** soman (150 μg/kg; van Helden et al. 1998), **B** tabun (384 μg/kg), **C** sarin (144 μg/kg), **D** VX (24 μg/kg), **E** parathion (12 mg/kg), and **F** vehicle ($n = 5–8$). The *solid columns* represent the rats treated with the organophosphate alone, the *hatched columns* represent the rats treated with 1 mg/kg i.m. CPA, and the *open columns* represent the rats treated with 2 mg/kg i.m. CPA 1 min following the organophosphate-poisoning. The emergence of clinical signs is expressed as the proportion of the total number of poisoned animals (*Chew* chewing, *Saliv* salivation, *Conv* convulsions, *R.Dist* respiratory distress)

In the placebo-treated groups the average survival times were 20.3 ± 2.2 min for tabun, 10.4 ± 0.9 min for sarin, 20.5 ± 3.6 min for VX and 94 ± 16.3 min for parathion, during which clinical symptoms associated with a nerve gas intoxication were observed. The vehicles used and 2 mg/kg CPA alone did not cause visible symptoms (Fig. 1F). Treatment with 1 or 2 mg/kg CPA upon tabun intoxication resulted in the complete suppression of all symptoms (Fig. 1B). In the case of sarin, administration of 1 mg/kg CPA attenuated the development of symptoms in 50% and the emergence of death in 67% of the animals (Fig. 1C). At a dose of 2 mg/kg CPA, the clinical symptoms were further diminished and all animals survived. In contrast, CPA turned out to be ineffective against VX and parathion (Fig. 1D, E). All cholinergic symptoms emerged and the mortality rate was unaffected. Only one CPA-treated parathion-intoxicated animal survived more than 24 h, albeit in a very poor condition. A repeated-dose regimen with 2 mg/kg CPA did not lead to survival of these animals either (data not shown). The rank order of the efficacy of CPA against the different nerve gasses was: tabun > soman > sarin > VX = parathion.

Microdialysis

In the untreated sarin-intoxicated animals, an 11-fold increase in extracellular ACh levels was observed within 10 min (Fig. 2). During this period, severe convulsions

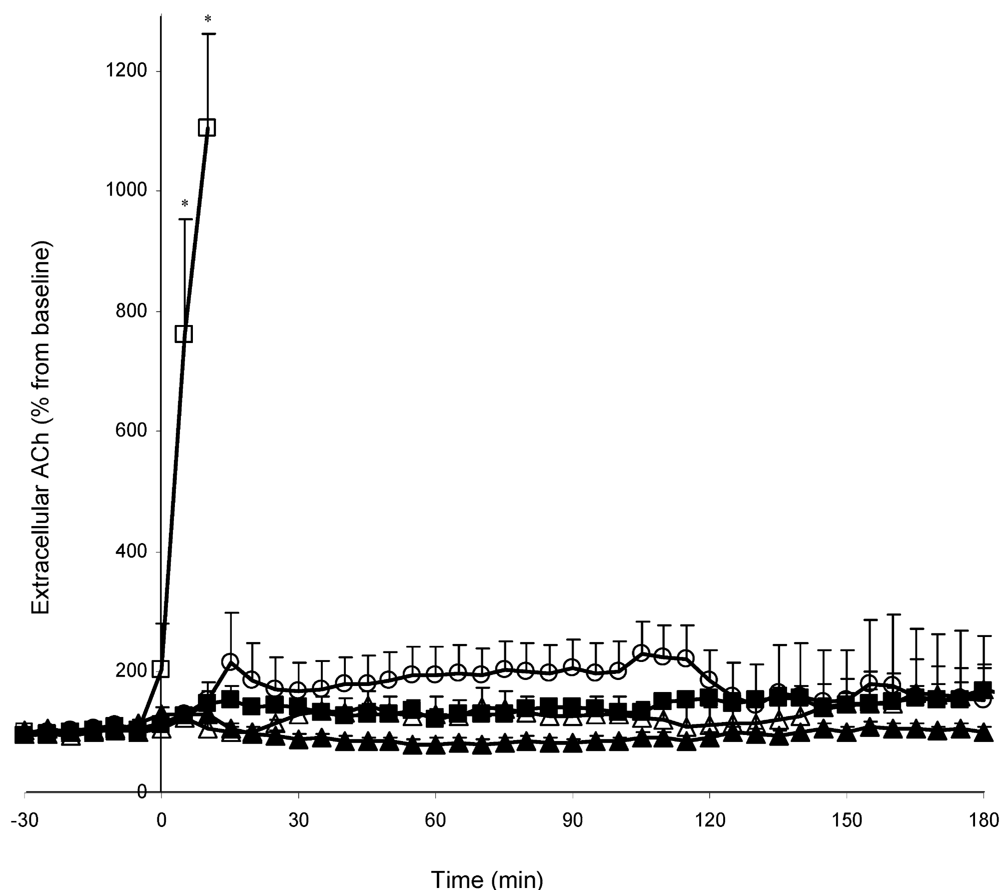

Fig. 2. The extracellular acetylcholine (ACh) levels in the striatum of sarin-intoxicated rats (144 μg/kg s.c.) treated with vehicle (*open squares*), or with 2 mg/kg N^6-cyclopentyladenosine (CPA) 1 min after the intoxication (*open circles*), or with 2 mg/kg CPA 2 min prior to the intoxication (*solid squares*), and the control rats injected with vehicle followed 1 min later with 2 mg/kg CPA (*solid triangles*), or with only vehicle (*open triangles*). The results (means + SEM, $n = 6$) are expressed as the change in ACh release compared with basal ACh levels before drugs were administrated. *$P < 0.05$, significantly different from the controls treated with vehicle only

Table 1. Average time (min; ± SEM) to the initial occurrence of the different clinical symptoms in the organophosphate-intoxicated animals in the microdialysis experiments. Sarin (144 μg/kg) and VX (24 μg/kg) were administered subcutaneously, whereas N^6-cyclopentyladenosine (CPA, 2 mg/kg) was injected intramuscularly 2 min before, or 1 min after exposure to the organophosphate

Treatment and order	No. of rats	Chewing	Convulsions	Respiratory distress	Death
Vehicle/Vehicle	6	–	–	–	–
Vehicle/CPA	6	–	–	–	–
Sarin/vehicle	6	2.8 ± 0.3	3.7 ± 0.5	5.2 ± 0.5	6.8 ± 0.9
Sarin/CPA	6	15.0 ± 3.2*	29.3 ± 7.2* (n = 4)	–	–
CPA/sarin	6	15.0 (n = 1)	22.0 (n = 1)	–	–
VX/vehicle	4	5.5 ± 1.0	8.8 ± 1.1	11.3 ± 1.0	16.8 ± 1.9
CPA/VX	4	12.0 ± 0.7**	16.8 ± 0.8**	19.0 ± 1.4**	24.3 ± 2.0*

*$P < 0.05$, **$P < 0.01$, statistically different from the untreated animals

and impaired movement of the animals were observed (Table 1). The average survival time was 6.8 ± 0.9 min. Animals treated with 2 mg/kg CPA at 1 min following the sarin intoxication displayed only a minor elevation in the extracellular ACh levels, which were not statistically significantly different from those of vehicle-treated rats. However, large inter-individual differences in the ACh profiles were apparent, which appeared to correlate with the intensity of the clinical symptoms, i.e. higher concentrations of extracellular ACh resulted in more severe symptoms. Pretreatment with 2 mg/kg CPA 2 min prior to the sarin intoxication, attenuated the clinical symptoms more effectively than in the post-treatment regimen (Table 1). The ACh release was normalized to that of the vehicle-treated animals (Fig. 2) and the inter-individual variability was decreased. Rats treated with CPA alone demonstrated somewhat reduced ACh levels compared with those of the vehicle-treated animals.

The corresponding brain AChE activity in untreated sarin-poisoned animals was strongly inhibited; only 1.1 ± 0.3% of the enzyme activity remained (Fig. 3). Striatal AChE activity in animals treated with CPA 1 min after sarin-exposure was reduced to 9.9 ± 3.7%, whereas pretreatment with 2 mg/kg CPA protected 60.2 ± 14.2% of the enzyme from being inactivated.

Figure 4 shows that ACh accumulation is evoked 18-fold relative to baseline values upon VX poisoning, which is in line with the results obtained in sarin-intoxicated rats. The average survival time of the vehicle-treated VX-exposed rats was 16.8 ± 1.9 min.

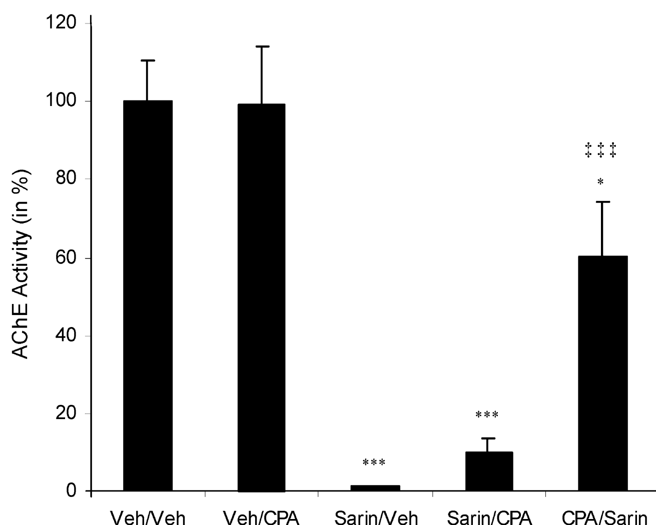

Fig. 3. The residual acetylcholinesterase (AChE) activity in the striatal tissue of sarin-poisoned animals (144 μg/kg s.c.) treated with vehicle (*Sarin/Veh*) or 2 mg/kg N^6-cyclopentyladenosine (CPA) 1 min after (*Sarin/CPA*) or 2 min prior (*CPA/Sarin*) to the exposure. The results (means + SEM, $n = 5$ or 6) are expressed as the change in AChE activity relative to the control animals treated with vehicle only (*Veh/Veh*). $^*P < 0.05$, $^{***}P < 0.001$, significantly different from the controls treated with vehicle only. $^{‡‡‡}P < 0.001$, significantly different from the placebo-treated sarin-intoxicated animals

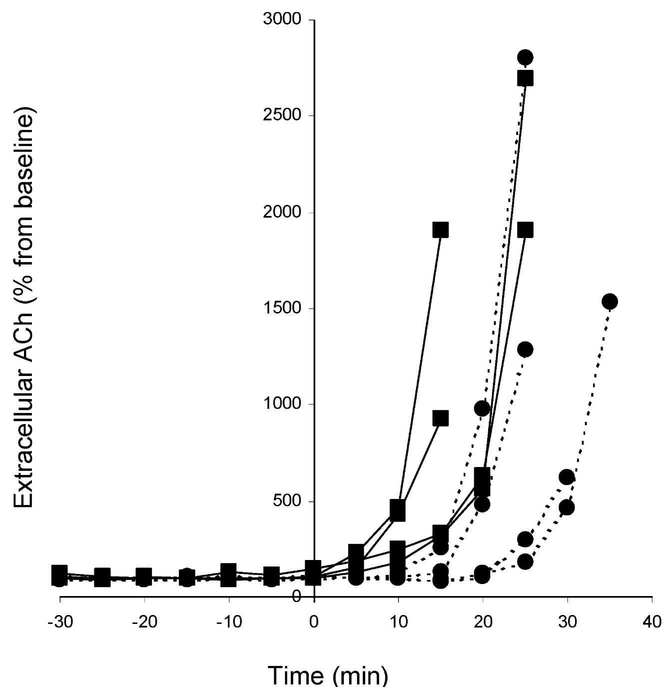

Fig. 4. The individual profiles of the extracellular acetylcholine (ACh) levels in the striatum of VX-intoxicated rats (24 μg/kg s.c.) treated with vehicle (*solid lines*, *solid squares*) or 2 mg/kg N^6-cyclopentyladenosine (CPA) 2 min prior to the intoxication (*broken lines*, *solid circles*). The results (means, $n = 4$) are expressed as the change in ACh release relative to the basal ACh levels before drugs were administrated

Pretreatment with 2 mg/kg CPA caused a delay in the accumulation of striatal ACh (Fig. 4), but did not ultimately affect the amount of ACh released. The delay in the rise of extracellular ACh is consistent with the observed 8 min delay in the emergence of the clinical signs and death (Table 1).

The residual striatal AChE activity in the VX-poisoned rats was 7.8 ± 5.3% and 12.7 ± 3.3% in placebo- and CPA-treated rats, respectively (Fig. 5). In the hippocampal tissue AChE was more severely inhibited; only 1.0 ± 0.2% and 1.1 ± 0.1% residual activity was found in those groups.

Discussion

The different OPs presently investigated produced typical parasympatic signs such as hypersecretion and muscular fasciculations, which reflect excessive cholinergic activity. However, the more disruptive and fatal toxic actions appeared to be mainly centrally mediated, since severe convulsive activity and general tremors always preceded incapacitation or death. This is consistent with the rapid increase in striatal ACh levels following soman- (van Helden et al. 1998), sarin- or VX-intoxication (not evaluated for tabun and parathion). These observations are in agreement with the current view on OP toxicity that the excessive build-up of central ACh and the subsequent overstimulation of cholinergic re-

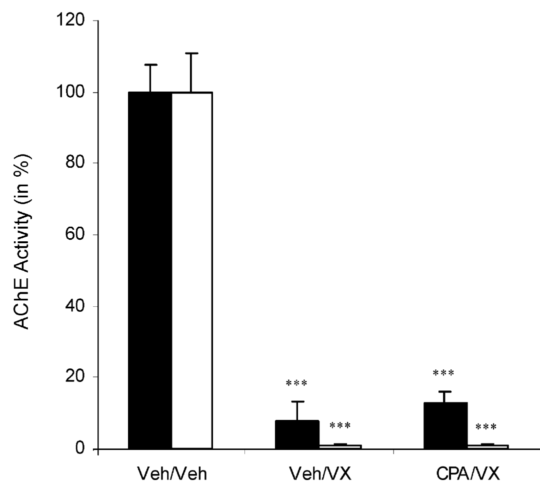

Fig. 5. The residual acetylcholinesterase (AChE) activity in the striatal (*solid bars*) and hippocampal (*open bars*) tissues of VX-poisoned animals (24 µg/kg s.c.), treated with vehicle (*Veh/VX*) or 2 mg/kg N^6-cyclopentyladenosine (CPA) 2 min prior to the exposure (*CPA/VX*). The results (means +SEM, $n=4$) are expressed as the change in AChE activity relative to the control animals, treated with vehicle only (*Veh/Veh*). $^{***}P<0.001$, significantly different from the controls treated with vehicle only

ceptors is considered as the crucial step in the initiation of convulsive activity, which eventually leads to profound neurotoxicity and death (Gupta et al. 1991; Kadar et al. 1995; McDonough et al. 1987; Shih and McDonough 1999; Solberg and Belkin 1997; van Helden and Bueters 1999).

The selective adenosine A_1 receptor agonist CPA protected rats against a lethal intoxication with tabun and sarin, which is in line with the therapeutic efficacy of CPA previously shown in soman-intoxicated rats (van Helden et al. 1998). Surprisingly, CPA did not protect against the toxic effects of VX and parathion. In contrast to intoxications by soman and sarin, the ACh accumulation in the brain was unaffected in the case of VX poisoning, supporting our hypothesis that reducing the release of ACh protects against OP-induced toxicity. Parathion was not evaluated in this respect.

There are at least two possible scenarios to explain why CPA managed to control the ACh accumulation in tabun-, sarin- and soman-poisoned animals and failed in VX- and parathion-poisoned animals. Besides their main AChE inhibitory properties, OPs have been shown to interact directly with muscarinic (Jett et al. 1991; Silveira et al. 1990), nicotinic (Rao et al. 1987) and glutamate receptors (Idriss et al. 1986), and to affect other transmitter systems via these receptors (Chebabo et al. 1999; Rocha et al. 1999). In the cases of VX and parathion, secondary toxicity pathways may obscure the CPA-mediated protection.

Another interesting explanation for the observed protection by CPA in the cases of tabun, sarin and soman, might be a delay in delivery of the toxic agents into the CNS. Adenosine A_1 receptor agonists have profound actions on the heart, resulting in severe bradycardia and hypotension (Olsen and Pearson 1990), which may influence the delivery of the OPs to the brain. Evidence for this assumption originates from the observations that CPA-pretreated rats that subsequently received VX showed an 8 min delay in the appearance of clinical signs (Table 1) and the accumulation of extracellular ACh in the striatum (Fig. 4), which may reflect changed delivery of VX into the brain. Moreover, the incomplete inhibition of the AChE activity in sarin-poisoned animals upon CPA administration points to a decreased bioavailability of the toxic agent in the brain, since the AChE activity is considered a direct and sensitive biomarker for OP-exposure (Nigg and Knaak 2000).

A delay in the delivery of the nerve agents to the brain has an immediate effect on their toxicity. The speed at which AChE is critically inhibited corresponds with the severity of the toxic signs; a slow reduction in AChE activity results in milder toxicity. This may be due to adaptational mechanisms such as modifications in the release of ACh and receptor desensitization (Gupta et al. 1986). Besides adaptational mechanisms, delayed delivery of these agents to the brain also reduces the final concentrations that reach the CNS. In the peripheral tissues tabun, sarin and soman are rapidly eliminated through irreversible interaction with various plasma proteins, such as carboxylesterase for which these nerve agents possess high affinity. Besides this, the nerve agent reacts with many non-specific binding sites due to its high reactivity. The ester moiety of these compounds is subject to chemical hydrolysis as well as enzymatic hydrolysis via various hydrolases. These enzymes are abundantly available in blood and liver (Nigg and Knaak 2000). Thus a prolonged presence of tabun, sarin and soman in the peripheral tissues results in greater elimination and, consequently, in a lower availability to the brain, which may ultimately contribute to the observed protection.

In VX-intoxicated animals the hippocampal AChE was completely inhibited, irrespective of CPA administration. The AChE activity in the striatum was not completely inhibited, which was also observed by Gupta et al. (1991). This indicates that, in VX- or parathion-exposed animals, delivery to the brain is not noticeably changed. This can presumably be attributed to higher chemical stability and slower pharmacokinetics in the body of VX and parathion than of sarin. VX has a high selectivity for AChE, is not hydrolyzed enzymatically and the ester moiety is chemically more stable than that of the other nerve agents. Parathion has much slower absorption, distribution and elimination characteristics than the nerve agents (Pena-Egido et al. 1988). Moreover, parathion is partially metabolized to paraoxon (Eigenberg et al. 1983), which is also a potent AChE inhibitor. Through these properties VX and parathion are more persistant in the body and a delay in delivery to the brain will not appreciably affect CNS availability as opposed to tabun, sarin and soman.

In conclusion, the present results demonstrate that the attenuation of the cholinergic signs and survival in tabun- and sarin-poisoned, and previously in

soman-poisoned rats, appear to be related to CPA-mediated reduction of extracellular ACh towards normal levels in the brain and to protection of AChE activity. The use of low efficacy adenosine A_1 agonists that inhibit the ACh release in the brain, but lack serious cardiovascular side effects, might reveal the contribution that the inhibition of ACh release makes to the observed protection.

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