Adenosine A₁ Receptor Agonist N⁶-Cyclopentyladenosine Affects the Inactivation of Acetylcholinesterase in Blood and Brain by Sarin

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ABSTRACT

The objective of the present study was to develop a kinetics of pharmacodynamics model to properly describe and investigate the in vivo interaction between the selective adenosine A₁ agonist N⁶-cyclopentyladenosine (CPA), acetylcholinesterase (AChE) in blood and brain, and the AChE-inhibitor sarin (isopropylmethylphosphonofluoridate). The direct interaction of CPA (2 μM) on the inhibition of AChE by sarin was studied in vitro in heparinized rat blood and in 10% (w/v) brain homogenate. CPA did not directly influence the sarin-mediated inactivation of AChE in either system. In sarin-poisoned (144 μg/kg s.c.) rats not treated with CPA, AChE was completely inactivated in blood and brain within 7 min. CPA (2 mg/kg i.m.) treatment, 1 min after sarin administration, caused a small delay in the inhibition of AChE in blood. Treatment with CPA, 2 min before sarin, protected the neuronal AChE partially from being inhibited, but not the enzyme localized in blood. With a dose-response-time model the proportion of the dose of sarin reaching the site of action was estimated to be 48 ± 12 or 13 ± 3% after CPA post- or pretreatment, respectively. A correlation between the residual AChE activity in the brain and the incidence of cholinergic symptoms could be established with logistic regression analysis: lower inhibition of AChE in the brain precluded the onset of critical symptoms. In conclusion, CPA affects the concentration of sarin reaching the site of action, which contributes to the protection previously observed in sarin-poisoned rats.

Sarin (isopropylmethylphosphonofluoridate) is an organophosphorous nerve agent that irreversibly inhibits the essential enzyme acetylcholinesterase (AChE), resulting in excessive amounts of acetylcholine (ACh) in cholinergic synapses. This cholinergic hyperactivity rapidly evolves into a more generalized neuroexcitability, which triggers seizure activity in susceptible brain areas (Kadar et al., 1995; Shih and McDonough, 1997; Van Helden and Bueters, 1999). Novel treatment strategies are currently under development, because the current therapy (atropine, oxime, and diazepam) seemed inadequate with respect to the suppression of the seizure activity and subsequent brain pathology in primates (Hayward et al., 1990; Van Helden et al., 1996; Shih and McDonough, 1997; Lallement et al., 1998). In this respect, a possible role for the adenosine A₁ receptor-mediated inhibition of ACh release in the brain was explored recently (Van Helden et al., 1998; Van Helden and Bueters, 1999; Bueters et al., 2002). This approach is aimed at the prevention or attenuation of ACh accumulation, thereby stifling the neurotoxic cascade in its birth.

Intracerebral application of a series of adenosine A₁ receptor agonists could effectively inhibit the ACh release (Bueters et al., 2000; Materi et al., et al., 2002). Microdialysis studies confirmed that accumulation of central ACh was attenuated upon CPA administration, which presumably explains the observed protection (Bueters et al., 2002).

However, some other observations do not easily fit with the conclusion that the protective effect of CPA is mediated via synaptic cholinergic modulation. In general, adenosine A₁ receptor agonists exhibit very poor blood-brain barrier transport properties, which makes it questionable whether sufficient concentrations of CPA reach the brain to achieve ade-
quate ACh inhibition (Pardridge et al., 1994; Kurokawa et al., 1996). Moreover, in sarin-intoxicated and CPA-treated rats, AChE activity in the brain was partially spared from being inhibited relative to untreated animals, indicating that CPA interferes with the inactivation of AChE by sarin (Bueters et al., 2002). CPA may achieve this by either directly blocking the interaction of sarin with the catalytic site of AChE, or indirectly by diminishing the amount of sarin reaching the central nervous system (CNS).

The aim of the present study was to characterize the interaction between CPA, sarin, and AChE. Hereeto, a kinetics of pharmacodynamics (K-PD) model has been developed to properly describe the in vivo three-way interaction between CPA, AChE in blood and brain, and the AChE inhibitor sarin. In addition, clinical symptoms associated with sarin poisoning were monitored examine a possible direct relationship between the severity of symptoms and the degree of AChE inhibition in blood and brain.

Materials and Methods

Animals. Male Wistar rats (−300 g; n = 67; Harlan B.V., Horst, The Netherlands) were housed with two or three animals per cage. Temperature was kept at 20 ± 1°C and relative humidity at 60 ± 5%, and a 12-h light-dark cycle was maintained (lights on at 7:00 AM). Acidified water and standard rodent chow (Teklad Global Diet; Harlan B.V.) were freely accessible. The Ethical Committee on Animal Experimentation of TNO approved all experiments described.

Determination of the Direct Effect of CPA on AChE Inactivation in Vitro. Rats were sacrificed (n = 3), blood was collected in heparinized glass pipes, and the brain was dissected out on ice. The brain tissue was homogenized [900 rpm, 10% (w/v) homogenate] in ice-cold TENT buffer, which consisted of 50 mM Tris, 5 mM EDTA, 1 M NaCl, and 1% v/v Triton X-100, pH 7.4 (Sigma-Aldrich B.V., Zwijndrecht, The Netherlands). CPA (2 μM; RBI, Inc., Zwijndrecht, The Netherlands) was incubated in 250-μl aliquots of blood or brain homogenate for 1 min at 37°C. Subsequently, sarin was added to the blood samples resulting in final concentrations of 0, 1.2, or 2.4 μM, or to brain homogenate samples, yielding final concentrations of 0, 14, and 28 nM. The mixtures were incubated for another minute. During the incubations steps the samples were gently shaken in an oscillating water bath. Finally, a 25-μl aliquot was drawn in duplicate and transferred into 250 μl of saponin solution (1%) or 250 μl of 50 mM phosphate buffer (PB) in case of blood or brain homogenate, respectively. Samples were immediately frozen and stored at −70°C until analysis.

After appropriate dilution in 50 mM PB the AChE activity was colorimetrically determined according to the method described previously by Ellman et al. (1961). Briefly, samples were preincubated with 4 mM 5,5′-dithio-bis-(2-nitrobenzoic acid) (Sigma-Aldrich B.V.) in 96-well plates for 5 min and the background signal was measured (λ = 415 nm). After addition of 25 μl of 10 mM acetylthiocholine iodide, the samples were incubated for 15 min at ambient temperature and the light absorption was determined. Effect was calculated as the percentage of the AChE activity with no drugs present.

Surgical Procedures. Rats were anesthetized with a single i.p. injection containing 0.675 ml/kg hypnorm (Janssen Pharmaceutica, Beersse, Belgium) and 0.675 ml/kg dormicum (Roche Nederland B.V., Mijdrecht, The Netherlands). An indwelling cannula was implanted in the right femoral artery for the serial collection of arterial blood samples. The cannula was filled with buffered saline (NPBI B.V., Emmer-Compascuum, The Netherlands) containing 20 U/ml of heparine (LEO Pharmaceutical Products B.V., Weesp, The Netherlands) and tunneled subcutaneously to the back of the neck. The incisions were sprayed with Nobecutan (Astra Meditec, Rijswijk, The Netherlands) to aid recovery. Rats were individually placed and allowed to recover 24 to 36 h from the surgical procedure.

Animal Experiments. Three groups of rats were injected according to the following treatment schedule group A, 144 μg/kg sarin s.c. followed by vehicle i.m. after 1 min (n = 20); group B, 144 μg/kg sarin s.c. followed by 2 mg/kg CPA i.m. after 1 min (n = 10); and group C, 2 mg/kg CPA i.m. followed by 144 μg/kg sarin s.c. after 2 min (n = 26).

CPA was administered in a mixture of 10% ethanol and 90% saline and sarin (provided by the Department of Chemistry, TNO Prins Maurits Laboratory) was dissolved in isopropylalcohol and further diluted in buffered saline directly before use.

Upon intoxication the rats were monitored and the following cholinergic symptoms were registered every minute:

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. Convulsion. Involuntary tensed movement in which the entire body is involved. The rat looks mentally dissociated from the environment and is refractory to stimulatory impulses.
3. Respiratory distress. Low respiratory rate and heavy breathing, often accompanied with some rattling, directing at obstruction in the throat.

During this observation period, a series of blood samples (50 μl) were drawn at predefined time intervals in 450 μl of saponine solution (1%) and immediately frozen in liquid nitrogen. The blood samples were drawn for 10 min with 1-min intervals every whole minute (0, 1, 2, etc.) or every half-minute (0, 0.5, 1.5, etc.) until the rats were sacrificed. In treatment group C, in a number of rats also at t = 15 min a sample was taken. Rats in group A were killed at 0, 1.5, 3, and 5 min and in group C at 0, 3, 5, 10, 15, and 180 min and the hippocampal tissues were dissected out to assay the AChE activity. After the hippocampi were homogenized (see previous section), they were centrifuged for 10 min at 1500g at 4°C (GS-6R; Beckman Coulter, Inc., Fullerton, CA). The supernatant was transferred in a clean tube, directly frozen in liquid nitrogen, and stored at −70°C until analysis.

Radiometric Determination of AChE Activity. After appropriate dilution, samples were assayed for AChE activity using a radiometric method described previously by Johnson et al. (1975). Briefly, acidified [3H]ACh iodide (37.0 MBq/5 ml ethanol; PerkinElmer Life Sciences, Boston, MA) was purified from [3H]choline by extraction with a mixture of toluene/isooamylic alcohol (9:1) followed by extraction with diethyl ether. Subsequently, 10 ml of a freshly prepared ACh perchlorate solution was added, which resulted in a final ACh concentration of 3 mM (60–80 μCi). To 50 μl of the samples, 500 μl of 50 mM PB containing 50 mM NaCl, 2 mM MgSO4, and 1% Triton X-100 and 25 μl of the labeled ACh were added, and the samples were incubated 20 min at ambient temperature. After the reaction was terminated with 100 μl of 10 M acetic acid, 4.5 ml of scintillation fluid (18 mM 2,5-diphenyloxazol, 0.25 mM 2,2′-p-phenylenebis(4-methyl-5-phenyloxazol and 11.0% (v/v) tert-my- lalcol in toluene) was added and the samples were firmly shaken. After both layers had separated, the AChE activity was counted. The activities were calculated with a calibration curve (15–7500 μU) on the basis of AChE from electric eel (Sigma-Aldrich B.V.) (r > 0.98; n = 7). The residual assay coefficient of variation for 75, 750, and 7500 μU were 1.8, and 8%, respectively (n = 5).

Dose-Response-Time Model. Because current analytical methods are insufficiently robust and sensitive to adequately determine free sarin concentrations in blood and brain, the residual AChE activity in blood and brain was determined. This is considered a sensitive biomarker for sarin exposure (Jokanovic and Maksimovic, 1997; Lotti, 1995; Nigg and Knaak, 2000). Subsequently, the profile of AChE inhibition by sarin versus time was characterized with a newly developed K-PD model, which extracts information about ki-
nomic parameters from the pharmacodynamic profiles (Fig. 1; Fisher and Wright, 1997; Gabrielson et al., 2000). In this model it is assumed that sarin is distributed upon dosing to a biophase (i.e., site of action) compartment with rate constant $k'$, from which it irreversibly inhibits AChE in both blood and brain. An irreversible response model described the AChE inhibition (Jusko, 1971). Because sarin stoichiometrically reacts with AChE, its concentration in the biophase compartment is directly linked to the irreversible response model of AChE. The rate of inactivation by sarin is then determined by the concentrations of sarin and AChE and a rate constant $k_{irr}$, which are different in blood and brain. To implement the influence of CPA on the distribution of sarin to the biophase compartment, the shift parameter was introduced. Under control conditions, i.e., in the absence of CPA, the value of this shift is 1. If CPA affects the distribution of sarin to the biophase, the shift parameter will deviate from 1: higher values indicate that a larger amount of sarin reaches the biophase compartments to inhibit AChE, and lower values mean that less sarin is transported to the biophase compartment. Given these interpretations, the biophase kinetics was modeled on the basis of two compartments, represented by the following equations

$$\frac{dC_{\text{dose}}}{dt} = D - k' \cdot C_{\text{dose}},$$

$$\frac{dC_{\text{bio}}}{dt} = \text{shift} \cdot k' \cdot C_{\text{dose}} - k' \cdot C_{\text{bio}},$$

in which $C_{\text{dose}}$ and $C_{\text{bio}}$ reflect the concentration of sarin at the dose site and in the biophase, respectively. $D$ is the dose of sarin administered, $k'$ represents the rate constant for sarin transport properties, and shift represents the steady-state partition coefficient.

The inactivation of AChE in blood and brain by sarin was modeled on the basis of an irreversible response model. The rate of change in the AChE activity was described according to the following equation:

$$\frac{d\text{AChE}}{dt} = -k_{irr} \cdot \text{AChE} \cdot C_{\text{bio}},$$

where $k_{irr}$ represents the elimination constant for the irreversible inhibition of AChE activity, AChE is the fraction of free AChE and $C_{\text{bio}}$ is the concentration of sarin in the biophase. Under physiological conditions, the rate of synthesis ($k_{syn}$) and degradation ($k_{degr}$) of AChE determine the total amount of AChE present, represented by the equation:

$$\text{AChE} = \frac{k_{syn}}{k_{degr}}.$$

The AChE activity in the untreated animals was defined 100%. In a population approach, the dose-response profiles of all individual rats in the different treatment groups were fitted simultaneously while explicitly taking into account the interindividual variability in the parameters as well as the residual variability. The interindividual variability of all parameters was modeled according to an exponential equation:

$$P_i = \theta \cdot \exp(\eta_i),$$

in which $\theta$ is the population estimate for parameter $P$, $P_i$ is the individual estimate, and $\eta_i$ the random deviation of $P_i$ from $P$. The values of $\eta_i$ are assumed to be independently normally distributed with mean zero and variance $\sigma^2$. The residual error was characterized according to the additive error model:

$$\text{AChE}_{mij} = \text{AChE}_{p ij} + \epsilon_{ij},$$

where $\text{AChE}_{p ij}$ is the $ij$th remaining enzyme activity for the $ij$th individual predicted by the model, $\text{AChE}_{mij}$ is the measured enzyme activity, and $\epsilon_{ij}$ accounts for the residual deviation in the model. The value of $\epsilon$ was assumed to be independently normally distributed with mean zero and variance $\sigma^2$. The model was implemented in the ADVAN9 subroutine in NONMEM (version V; NONMEM project group, University of California-San Francisco, San Francisco, CA). The first-order estimation method was used to estimate the values of the population $\theta$, $\sigma^2$, and $\omega^2$.

**Logistic Regression Analysis.** The relationship between the residual AChE activity in blood and hippocampus and the onset of occurrence of the three cholinergic symptoms studied (chewing, convulsions, and respiratory distress) was investigated. The appearance of a symptom was marked as a positive response; the absence of a symptom during the sarin exposure was marked as a negative response. Both negative and positive responses were related to the residual AChE activity. The data obtained from the three treatment groups were pooled and analyzed with linear logistic regression. The probability of a response was investigated based upon a linear logistic model governing the probability $p_i$ of the $ij$th response being 1. The log of $p_i$ is given by the following equation:

$$\logit(p_i) = \theta_1 + \theta_2 \cdot \text{AChE}_i,$$

where $\theta_1$ and $\theta_2$ represent the fixed effect parameters for slope and intercept and $\text{AChE}_i$ is the value of the residual AChE activity. The intercept is given by the $-\log$ of the EC50 value of the response times the slope parameter. The probability is then as follows:

$$p_i = \frac{e^{\logit(p_i)}}{1 + e^{\logit(p_i)}}.$$

The appearances of the different cholinergic signs were fitted simultaneously while taking only residual variability into account, i.e., interindividual random effects were not included in the model. The reason to exclude interindividual variation was because only one observation per clinical sign per individual rat was made. Yano et al. (2001) showed that in such a case estimates are not improved by including an interindividual error model. Similar to the previously described model, the residual variability was characterized by an additive error model (eq. 6). The values of the population $\theta$ and $\omega^2$ were estimated with NONMEM and individual parameter estimates were obtained in a Bayesian post hoc step.

**Statistical Analysis.** Goodness-of-fit was analyzed using visual inspection, objective function, and assessment of parameter correlation. Statistical analysis was performed using one-way analysis of variance followed by the Student Newman-Keuls test, whenever
appropriate. All data are represented as mean ± S.E.M. and differences were considered significant for $P$ values < 0.05.

**Results**

**Direct Effect of CPA on the AChE Inactivation by Sarin in Vitro.** Increasing concentrations of sarin dose-dependently diminished the AChE activity in both blood and brain preparations, regardless of the presence of 2 μM CPA (Fig. 2). A 100-fold higher concentration of sarin was required to inactivate a similar amount of AChE in blood compared with the brain tissue.

**Effect of CPA on the Distribution of Sarin in Vivo.** Fig. 3 shows the observed, predicted population, and individual AChE activity-time profiles in blood for the three treatment groups, based upon the K-PD model (Fig. 1). Population parameter estimates are summarized in Table 1. From each rat a blood sample was drawn before drug administration, of which the AChE activity was set at 100%. Control groups that received only vehicle treatment ($n = 3$) or vehicle followed by 2 mg/kg CPA ($n = 5$) showed no loss in AChE activity in blood and brain tissue (data not shown). Directly upon subcutaneous administration of sarin, a steep decrease in AChE activity was apparent in rats that did not receive CPA treatment (Fig. 3A; $n = 20$, group A). Within 4 min, the AChE activity was abolished to 0.6 ± 0.3% of control value. CPA treatment shortly after sarin was given, resulted in a small delay in inactivation of the AChE activity, but the AChE was still maximally inhibited (Fig. 3B; $n = 10$, group B). Treatment with CPA 2 min before sarin administration led to a more pronounced rightward shift of the inactivation curve of AChE in time (Fig. 3C; $n = 26$, group C). Moreover, in several individual animals some AChE activity remained after 15 min, resulting in an average residual AChE activity of 10.3 ± 4.7%. In this treatment group, one rat died after 10 min, which was excluded from further analyses. In Fig. 3D, the population profiles are summarized, in which the rightward shift as a consequence of the CPA treatment is clearly demonstrated.

The corresponding AChE activities of the individual rats (observed and predicted) in the hippocampus are shown in Fig. 4. The AChE activity in rats that did not receive CPA treatment declined maximally within 7 min to 0.7 ± 0.3% of control values. Upon CPA administration before sarin, AChE...
was partially saved from inhibition by sarin after 180 min; 34.5 ± 0.8% AChE activity remained. All individual profiles were adequately fitted with the dose-time-response model. The value of $k_{2_{\text{irr}}}$ was estimated to be 0.06 ± 0.01 min$^{-1}$ (Table 1). This estimation was based on the data from treatment group A and C only. Moreover, estimates for the CPA-mediated effect on the distribution of sarin to the biophase were obtained, reflected by the shift parameter (Table 1). For groups B and C this parameter was 0.48 ± 0.12 and 0.13 ± 0.03, respectively. This means that the dose of sarin reaching the site of action was 48 ± 12 or 13 ± 3% after CPA post- or pretreatment, respectively.

**Relationship between Symptomatology and AChE Activity.** During the experiments described above, the occurrence of chewing, convulsions, and respiratory distress were monitored to obtain a relationship between the amount of AChE inhibited and the visible condition of the rats. The observed clinical signs from the three treatment groups were pooled and related to the residual AChE activity in blood and brain tissue with a linear logistic regression model (Fig. 5). The parameter estimates are listed in Table 2. Figure 5 shows that the AChE activity in blood does not correlate with the emergence of cholinergic symptoms. With complete AChE inhibition the probability of response is approximately 60%. In contrast, the residual AChE activity in the brain was very well correlated with the severity of symptoms. The critical AChE activity is approximately 7%; lower AChE activities are likely to result in convulsions and respiratory distress.

### Discussion

The aim of the present study was to characterize the interactions between CPA, sarin, and AChE and to examine whether these interactions could explain the observed protection by CPA against such poisoning. Hereto, a K-PD model has been developed to properly describe the in vivo three-way interaction between CPA, AChE in blood and brain, and sarin. From the in vitro results, it seemed that CPA did not block the catalytic site of AChE for sarin. However, CPA altered the transport of sarin from the injection site to the biophase, which was more pronounced when CPA was present at the moment of sarin administration. The effective dose of sarin reaching the biophase was markedly reduced upon CPA presence, resulting in a smaller inactivated fraction of AChE, specifically in the brain, where sarin exerts its main toxicological effects (Gupta et al., 1991; Kadar et al., 1995; Bueters et al., 2002). This spared fraction of AChE was sufficient to explain the absence of symptoms associated with severe AChE inhibition.

![Fig. 4. A and B, individual time-response profiles for the inhibition of AChE in the brain by sarin for the treatment groups A and C (A: $t = 0$, 144 μg/kg sarin, $t = -1$, vehicle; C: $t = -2$, 2 mg/kg CPA, $t = 0$, 144 μg/kg sarin). The observed AChE activities (symbols), the individual predictions (dotted lines), and the population predictions (thick lines) are depicted. C, summarizes A and B to clearly demonstrate the influence of pretreatment with CPA (2 mg/kg) on the sarin-mediated inactivation of AChE in brain.](image-url)
Relationship between AChE and the Symptomatology. Recently, analytical methods have been developed to determine sarin directly in plasma (D’Agostino et al., 1999, 2001; Spruit et al., 2000, 2001). However, these assays are neither sensitive nor robust enough to detect concentrations of free sarin in brain tissues. Therefore, the AChE activity is still used as a sensitive biomarker for sarin exposure (Lotti, 1995; Jokanovic and Maksimovic, 1997; Nigg and Knaak, 2000). The isoform of AChE on red blood cells is similar to the enzyme present in the synapses, and some therefore consider erythrocyte AChE inhibition a biomarker for organophosphate toxicity (Holstege et al., 1997; Jokanovic and Maksimovic, 1997). The results of the present study clearly demonstrate that this can be misleading and great care should be taken in extrapolating the amount of inhibited AChE in blood to that in the CNS. In all treatment groups, AChE in the blood is maximally or nearly maximally inhibited, whereas large differences exist between the remaining enzyme activity in the brain. Moreover, the AChE activity in blood seems to be a very poor predictor for the severity of the intoxication (Fig. 5). Therefore, inhibition of AChE activity in blood should only be interpreted as an indicator for exposure and not for toxicity. In contrast, the enzyme inhibition in the brain correlates very well with the severity of the intoxication. Neuronal AChE levels greater than 10 to 15% will still investigate the impact of CPA on the distribution pattern of sarin. The model was adopted from Jusko (1971), who applied it to quantitatively predict the efficacy of chemotherapeutic agents. To reduce the number of parameters, the biophase kinetics of sarin was described with a single rate constant, i.e., it was assumed that the distribution to the effect site was equal to the elimination from this site (Gabrielsson et al., 2000).

The turnover constants for the synthesis and metabolism of AChE (i.e., $k_{in}$ and $k_{out}$) were not incorporated in the model for two reasons. They turned out to be negligible relative to the value of $0.13 \pm 0.02$ and $0.06 \pm 0.01 \text{ min}^{-1}$ for the elimination rates $k_{1\text{irr}}$ and $k_{2\text{irr}}$. The synthesis and elimination constants of AChE are estimated to be $3.2 \cdot 10^{-5} \text{ nmol/min}$ and $1.7 \cdot 10^{-3} \text{ min}^{-1}$ in rat plasma and $2.3 \cdot 10^{-5} \text{ nmol/min}$ and $1.7 \cdot 10^{-4} \text{ min}^{-1}$ in rat brain, respectively (Wentholt et al., 1974; Gearhart et al., 1994). This directly implies that the shape of the inhibitory profile is only dependent on the interaction of sarin with the AChE, reflected by the elimination rate constant $k_{2\text{irr}}$ and the concentration of sarin at the site of action. Second, no information about the $k_{out}$ could be derived from the first part of the curve. For that, the recovery of the AChE activity in blood and brain had to be followed, which was beyond our scope and ethically unde-

**TABLE 2**

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<th>Symptom</th>
<th>Slope</th>
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| AChE activity in blood
| Chewing         | 4.1 ± 2.3 | 1.8 ± 0.4 |
| Convolusions     | 3.7 ± 1.7 | 1.4 ± 0.2 |
| Respiratory distress | 1.8 ± 0.6 | 1.6 ± 0.3 |
| AChE activity in brain
| Chewing         | 3.7 ± 3.4 | 15.4 ± 8.8 |
| Convolusions     | 9.5 ± 0.7 | 7.8 ± 0.1 |
| Respiratory distress | 7.4 ± 1.5 | 5.3 ± 1.3 |
sired for the animals, because this may take some weeks (Tripathi and Dewey, 1989; Lotti, 1992).

Our K-PD model adequately described the obtained individual profiles and changes in these profiles as a consequence of CPA administration. Because CPA did not affect the direct interaction between sarin and AChE in vitro, the elimination rate constant $k_{1\text{irr}}$, which reflects this interaction, among the different treatment groups is constant. This leaves only one factor responsible for the observed change in the slope of the inhibitory profiles: the concentration of sarin at the site of action. This loss in transport of sarin to the biophase was quantitatively estimated via the introduced shift parameter representing the CPA influence. This resulted in a 52 and 87% loss of the total given dose after CPA post- and pretreatment, respectively.

The difference between the elimination constants ($k_{1\text{irr}}$ and $k_{2\text{irr}}$) of AChE in blood and brain is probably caused by the fact that the inactivation of AChE in blood and brain is driven by one biophase concentration. It seems logical to differentiate between a blood and CNS compartment, for instance, to be able to estimate hysteresis of the response in the brain. We have tried to introduce such a compartment but were not able to characterize it adequately. Therefore, this model is successful in describing, linking and quantifying the interaction of AChE inhibition in blood and brain tissue by sarin with and without the presence of CPA, but has its limits in terms of the exact mechanism of action.

However, the profound adenosine $A_1$ receptor-mediated hypotension and bradycardia by CPA (Mathot et al., 1994; Van Schaick et al., 1998) may explain the loss in the amount of sarin reaching the biophase. In the peripheral tissues sarin is rapidly eliminated through irreversible interaction with various plasma proteins such as carboxylesterase as well as many non-specific binding sites due to its high reactivity. Moreover, sarin is subject to enzymatic hydrolysis. These enzymes are abundantly available in blood and liver (Nigg and Knaak, 2000). Thus, a prolonged presence of sarin in the peripheral tissues as a result of the CPA-induced inhibition will lead to greater elimination of sarin and consequently to lower amounts reaching the brain.

In conclusion, the relationship between the emergence of critical symptoms and the residual AChE in the brain clearly demonstrated that the sarin-induced toxicity is centrally mediated. The developed K-PD model could characterize the influence of CPA on the distribution of sarin from the injection site to the AChE in blood and brain based upon the residual AChE activities in these tissues. This led to the insight that the cardiovascular actions of CPA have highly contributed to its previously observed protection against sarin poisoning.

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References


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