REFERENCES

1. T. P. M. Akerboom, M. Bilzer and H. Sies, *J. biol. Chem.* **257**, 4248 (1982).
2. P. A. Krieter, D. M. Ziegler, K. E. Hill and R. F. Burk, *Molec. Pharmac.* **26**, 122 (1984).
3. Y. Xia, K. E. Hill and R. F. Burk, *J. Nutr.* **115**, 733 (1985).
4. R. N. McCoy, M. A. Ayon, K. E. Hill, J. H. Stein and R. F. Burk, *Kidney Int.* **29**, 307 (1986).
5. P. L. Wendell, *Biochem. J.* **117**, 661 (1970).
6. P. A. Krieter, D. M. Ziegler, K. E. Hill and R. F. Burk, *Biochem. Pharmac.* **34**, 955 (1985).

Biochemical Pharmacology, Vol. 37, No. 5, pp. 969–970, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Ketamine protects acetylcholinesterase against *in vitro* inhibition by sarin

(*Received* 8 *July* 1987; *accepted* 17 *September* 1987)

Ketamine (2-(*o*-chlorophenyl)-2-methylamino-cyclohexone) is a drug that can be used for anesthetic, analgetic and sedative purposes. Several mechanisms have been proposed for this versatile substance [1]. As anesthetics in general, ketamine induces changes in the membrane fluidity, e.g. in synaptosomes and mitochondria [2]. It also has ion channel blocking properties [3]. Furthermore, several central nervous transmitter systems, including the cholinergic one, are affected during ketamine anesthesia [1].

Our interest in this drug and its interaction with the cholinergic system was raised during a study on pigs, intoxicated by an anticholinesterase compound [4]. Animals were anesthetized either by ketamine or by sodium pentobarbital. We observed that the ketamine-treated pigs tolerated more of the anticholinesterase (soman) than did pigs pretreated with pentobarbital. This difference in sensitivity towards the cholinesterase inhibitor could mean that pentobarbital potentiates the toxicity or that ketamine reduces the toxicity or both. The first hypothesis finds some support from a work by Clement [5], who claims that pentobarbital enhances the toxic effect of soman in mice. The second hypothesis is supported by recent findings by Klemm [3]. He observed that ketamine protects against intoxication by organophosphates, and he suggested that this protection is due to the ionic channel blocking properties.

It is, however, possible that such a protection could at least partly be mediated by protection of the target enzyme acetylcholinesterase (EC 3.1.1.7), as it has been shown that ketamine is a reversible inhibitor of this enzyme, both *in vitro* and *in vivo* [6].

The interactions between ketamine, acetylcholinesterase from bovine brain and the potent organophosphorous inhibitor sarin (isopropyl methylphosphonofluoridate) have now been studied. The study also includes results on the influence of ketamine on reactivation and dealkylation ("aging") of sarin-inhibited enzyme, i.e. reactions of importance for the therapeutic countermeasures against organophosphate intoxication.

Materials and methods

Ketamine hydrochloride was a gift from Parke Davis Co. and HI-6 dichloride from Dr J. Clement, DRES, Canada. Sarin was synthesized at the Chemistry Department of this institute.

Acetylcholinesterase was purified from bovine brain with affinity chromatography, as described recently [7]. The preparation had a specific activity of 1.5 μkat mg^{-1}. Enzyme activity was measured by Ellman's procedure [8], using 1 mM acetylthiocholine as substrate.

Inhibition by ketamine. The reversible inhibition constants were determined, using ketamine in the concentration range 0.5–2 mM and varying the substrate concentration between 0.1 and 0.9 mM. These experiments were performed at 22°, in 0.1 M sodium phosphate buffer, 0.1% Triton X-100, pH 8.0. Calculations were based on Eadie–Hofstee plots.

Sarin inhibition. Enzyme, sarin and ketamine were incubated in 0.1 M sodium phosphate buffer, 0.1% Triton X-100, pH 7.4 at 22°. Sarin concentration was varied between 0.05 and 0.5 μM and ketamine concentration between 0.5 and 5 mM; no ketamine in control experiments. Aliquots from the incubation media were taken in a time series, usually every second minute. The irreversible inhibition constant, k_i, was determined as the rate constant, obtained by linear regression, divided by the sarin concentration used.

Aging rate constant, k_a, *and reactivation efficacy.* Enzyme was inhibited by 0.5 μM sarin for 5 min at pH 7.4. Excess sarin was removed by gel filtration on a Pharmacia PD-10 column (Sephadex G-25 gel), equilibrated with 0.067 M sodium phosphate buffer, 0.1% Triton X-100, pH 6.9. Uninhibited enzyme was treated in the same way. The enzyme solutions thus obtained, denoted EI and E respectively, were used for the aging and reactivation experiments.

For the aging rate experiments, two 0.4 ml samples of E and EI, respectively, were transferred to tubes containing 1.6 ml 0.067 M sodium phosphate buffer, with 0.1% Triton X-100, pH 6.9, with and without 5 mM ketamine, at 37°. The experimental conditions (ionic strength, pH and temperature) were chosen to give a rather rapid aging. From these tubes 2 × 0.1 ml aliquots were withdrawn in a time series, t = 0–5 hr, and transferred to tubes containing 0.3 ml 0.1 M sodium phosphate buffer, 0.1% Triton X-100, pH 7.4, with and without 0.075 mM HI-6. Enzymatic activity was measured after 30 min of reactivation. Percentage reactivation and the rate constant of aging were calculated according to Keijer and coworkers [9].

Reactivation experiments. These experiments were performed at pH 7.4, 0.1 M sodium phosphate buffer, 0.1% Triton X-100, obtained by diluting E and EI 20 times. The oxime (HI-6) concentration was varied between 0.01 and 0.075 mM. The series with ketamine were done at a concentration of 5 mM. Proper controls such as activity of E and EI in the absence of oximes were also run. Aliquots were withdrawn in time series, usually t = 5–400 min, the activity measured and percentage reactivation calculated as described above. The rate constant, k_{obs}, was calculated from Guggenheim plots, ln (% react$_{t=\infty}$ − % react$_t$) vs t, where reactivation at t$_\infty$ was estimated as the value obtained after 24 hr. The bimolecular reaction rate constant, k_2/K_d, which reflects reactivation efficacy, was obtained as the slope of a plot $1/k_{obs}$ versus 1/(HI-6).

Results and discussion

We could confirm previously reported results that ketamine inhibits acetylcholinesterase, with a mixed type of

Table 1. The influence of ketamine on inhibition of acetylcholinesterase by sarin

	Ketamine concentration (mM)				
	0	0.5	1	2	5
k_i (sarin) (10^6 M^{-1} min^{-1})	2.86	1.88	1.61	0.79	0.46
(SD)	0.17	0.49	0.32	0.08	0.08
(N)	(7)	(4)	(5)	(4)	(5)

inhibition [5]. The inhibition constants were determined to $K_i = 0.52$ mM (SD 0.098) and $K_i' = 3.3$ mM (SD 0.67).

An interaction with the active site may result in a protective effect against the irreversible inhibitor sarin, in analogy with the action of several other reversible, competitive inhibitors. This was also found to be true for ketamine, as shown in Table 1. It protected against the irreversible inhibitor in a concentration-dependent manner, but the data could not be described by the most simple kinetics, $k_{obsd} = k_i/(1 + [\text{ketamine}]/K_i)$. The observed k_is were, for all concentrations of ketamine, higher than calculated from such a simple interaction only at the active site.

The influence of ketamine on aging and reactivation was studied at only one and a rather high (5 mM) concentration. From Table 2 it can be concluded that ketamine retarded both the rate of reactivation and, to a lesser extent, the rate of aging. A possible explanation for these effects is that a conformational change of the phosphonylated enzyme is induced by ketamine-binding.

Table 2. The influence of 5 mM ketamine on the aging rate constant (k_a) and the reactivation efficiency (k_2/K_d) by HI-6 of sarin-inhibited acetylcholinesterase

	k_a (min^{-1})	k_2/K_d (10^3 M^{-1} min^{-1})
Control	0.260	1.28
SD	0.008	0.21
(N)	(3)	(3)
Ketamine	0.197	0.61
SD	0.028	0.12
(N)	(3)	(3)

Mazzanti *et al.* have recently shown that the local concentration of ketamine in cholinergic synaptical membranes during anesthesia is high [2]. Their results on membrane fluidity changes and effects on acetylcholinesterase activity in combination with the results presented here give some support for the hypothesis of ketamine protection against organophosphate poisoning. Current research on acetylcholinesterase levels in ketamine-anesthetized organophosphate-intoxicated animals will throw more light on this issue. The question whether ketamine facilitates (by reducing the rate of aging), aggravates (by reducing the efficiency of reactivation by oximes) or has no effect at all on the therapeutic countermeasures in the *in vivo* situation also deserves further investigations.

Division of Experimental Medicine
National Defence Research Institute, Dept. 4
S-901 82 Umeå, Sweden

GERTRUD PUU

REFERENCES

1. P. F. White, W. L. Way and A. J. Trevor, *Anaest.* **56**, 119 (1982).
2. L. Mazzanti, A. Pastuszko and G. Lenaz, *Biochim. biophys. Acta* **861**, 105 (1986).
3. W. R. Klemm, *Toxic. Lett.* **25**, 307 (1985).
4. S.-Å. Persson, G. Cassel, L. Karlsson, R. Larsson, Å. Nilsson, G. Puu and T. Jeneskog, *Proceedings Second International Symposium on Protection against Chemical Warfare Agents, FOA Report C40228,* 365 (1986).
5. J. Clement, *Biochem. Pharmac.* **33**, 683 (1984).
6. M. L. Cohen, S. L. Chan, H. N. Bhargava and A. J. Trevor, *Biochem. Pharmac.* **23**, 1647 (1974).
7. G. Puu, E. Artursson and G. Bucht, *Biochem. Pharmac.* **35**, 1505 (1986).
8. G. L. Ellman, K. D. Courtney, V. Andres and R. M. Featherstone, *Biochem. Pharmac.* **7**, 88 (1961).
9. J. H. Keijer, G. Z. Wolring and L. P. A. de Jong, *Biochim. biophys. Acta* **334**, 146 (1974).

Biochemical Pharmacology, Vol. 37, No. 5, pp. 970–973, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

Activation of the carcinogen, 5-hydroxymethylchrysene, to the mutagenic sulphate ester by mouse skin sulphotransferase

(*Received* 23 *April* 1987; *accepted* 29 *September* 1987)

5-Methylchrysene (5-MCR), a well known environmental carcinogen found in tobacco smoke, shows carcinogenicity towards adult mouse skin [1] as well as towards newborn mouse skin and lung [2]. Metabolic oxidation of 5-MCR gave 5-hydroxymethylchrysene (5-HCR) as a major metabolite, dihydrodiols, and phenols in mouse skin *in vivo* [3] and in the livers of mice and rats *in vitro* [3, 4]. 5-HCR had as high carcinogenicity to mouse skin as 5-MCR, suggesting it to be one of proximate metabolites in the mouse skin [5]. The carcinogen 5-HCR was demonstrated to be activated to a potent mutagenic metabolite, 5-HCR sulphate, by rat liver cytosolic sulphotransferase in the presence of a 3′-phosphoadenosine 5′-phosphosulphate (PAPS)-generating system [6]. The active sulphate bound covalently to calf thymus DNA at pH 7.4 through its 5-methylene carbon with loss of a sulphate anion and was inactivated to a non-mutagenic glutathione (GSH) conjugate by rat liver cytosolic GSH transferase in the presence of GSH [7].

However, nothing is known of whether 5-HCR is biotransformed to the active sulphate in the skin. Little information is available as yet for the metabolic conjugation of hydrophobic alcohols to the corresponding sulphates in the skin. To our knowledge, dehydroepiandrosterone (DHA) sulphate formation from DHA in the human skin was the first instance for these informations [8]. Sulphation of steroid alcohols has recently been demonstrated with