Biochemical Pharmacology, Vol. 35, No. 2, pp. 347–349, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

SHORT COMMUNICATIONS

Reactivation of Sarin- or Soman-phosphonylated human acetylcholinesterase by bis-pyridinium mono-oximes

(*Received* 5 *February* 1985; *accepted* 12 *June* 1985)

Bis-pyridinium mono-oxims are thought to be possible antidotes to Soman intoxication [1, 2]. Although reactivation of dealkylated phosphonylated acetylcholinesterase (AChE, EC 3.1.1.7) remains a problem, they have shed light on the treatment of Soman poisoning. In this paper, the reactivating potencies of 1-(2-hydroxyiminomethyl-1-pyridinio)-3-(4-amino carbonyl-1-pyridinio)-2-oxapropane dichloride (HI-6) and 1-(2-hydroxyiminomethyl-1-pyridinio)-3-(3-cyclohexyl carbonyl-1-pyridinio)-2-oxapropane dichloride (HGG-42) on aged as well as non-aged phosphonylated human and rat cholinesterases were examined.

Human blood was collected from normal adults via vein acupuncture. Red cells were washed with and suspended in 310 milliosmolar (mOsM) sodium phosphate buffer [3], pH 5.3, 7.2 or 10 (adjusted with NaOH). Human brain from a 60-year-old man who died of diabetes, and human diaphragm and intercostal muscles from a 68-year-old man who died of acinous carcinoma of the prostate gland were taken several hours after death. Tissue samples were stored at −20°, pulverized in liquid nitrogen, and homogenized in buffer seprately before use. Blood and tissues from male Wistar albino rats, weighing 150–250 g, were also used. Soman and Sarin of more than 95% purity were prepared in this Institute. Bis-pyridinium mono-oximes and other reagents were all of an analytical grade.

Non-aged Sarin-phosphonylated cholinesterase. Aliquots (0.1 ml) of a 1:3 (v/v) human red cell suspension in 310 mOsM phosphate buffer (pH 7.2) were incubated with equal volumes of 1 μM sarin at 37° for 10 min. Red cells were washed twice with buffer and resuspended in a final volume of 0.5 ml. The same volumes of 1 mM mono-oximes were added to separate tubes. After incubation for 30 min, red cells were washed and resuspended as before. Cholinesterase activity was then determined using a microassay method modified from Hestrin [4]. Blanks for the activity of the enzyme (E), for the activity of the enzyme incubated with oxime (ER), and for the activity of the inhibited enzyme (EI) were run in a similar manner. The percentage of reactivation obtained was calculated according to Gilbert *et al.* [5]. As shown in Table 1A, the percentages of reactivation for human non-aged Sarin-phosphonylated red cell AChE induced by HI-6 and HGG-42, when used at a final concentration of 0.5 mM, were 92 and 83, respectively, comparable to 98% of 2-pyridinium aldoxime methochloride (2-PAM·Cl) which was run presumably as a control of reactivator.

Aged Sarin-phosphonylated cholinesterase. To facilitate aging of Sarin-phosphonylated cholinesterase, red cells were incubated at 37°, pH 5.3, for 3.5 hr with an equal volume of 40 nM Sarin, then washed with buffer (pH 7.2), and resuspended. Drugs were added separately to a final concentration of 0.33 mM. After incubation and washings, enzyme activity was assayed as before. It can be seen in Table 1B that the reactivation percentages of these three drugs were all around 20%, which may have been due to the non-aged portions of Sarin-phosphonylated cholinesterase.

Non-aged Soman-phosphonylated cholinesterase. For the sake of retardation of Soman-phosphonylated cholinesterase, inhibition was carried out at 0°, pH 10. A 1:3 red cell suspension (0.1 ml) was incubated with an equal volume of 40 nM soman, washed twice with buffer (pH 10), and resuspended in 0.5 ml of it. Aliquots (0.1 ml) of 2 mM drugs were added separately. Reactivation proceeded at 26° for 30 min. Red cells were then washed with buffer (pH 7.2) and resuspended, and enzyme activity was estimated. Table 2A shows that HI-6, HGG-42 and 2-PAM, at final concentrations of 0.33 mM, reactivated human non-aged Soman-phosphonylated red cell AChE 67, 31 and 22% respectively. Among these oximes, HI-6 was the most potent reactivator. The percentage of reactivation induced

Table 1. Reactivation of Sarin-phosphonylated human cholinesterase

Tissue	Drug	Cholinesterase activity (%) ER	Cholinesterase activity (%) EI	Reactivation (%)
		(A)*		
Red cell	HI-6	91 ± 11	5 ± 3	92 ± 5
	HGG-42	96 ± 1	5 ± 3	83 ± 8
	2-PAM	97 ± 6	5 ± 3	98 ± 9
		(B)†		
Red cell	HI-6	93 ± 4	4 ± 3	23 ± 4
	HGG-42	89 ± 2	4 ± 3	22 ± 4
	2-PAM	93 ± 5	4 ± 3	23 ± 1

* (A) Human ChEs; inhibition, 0.5 μM Sarin, 37°, pH 7.2, for 10 min; reactivation, 0.5 mM drugs, for 30 min at the same conditions; enzyme assay, 37°, pH 7.2, for 30 min; mean ± S.D., average of three determinations in duplicate. ER, activity of the enzyme incubated with oximes; EI, activity of the inhibited enzyme.

† (B) Human ChEs; inhibition, 20 nM Sarin, aged at 37°, pH 5.3, for 3.5 hr; reactivation, 0.33 mM drugs, 37°, pH 7.2, for 30 min; enzyme assay, 37°, pH 7.2, for 30 min; mean ± S.D., average of two determinations in duplicate.

by HI-6 was comparable to those obtained by de Jong and Worling [6, 7]. The red cell cholinesterase activities of drug controls (ER) in Table 2A are all lower than those in Tables 1A and B and 2B. As enzyme activity after incubation for 30 min at pH 10 did not decrease (data not listed), we assume that the drugs may inhibit the enzyme activity more at pH 10 than at pH 7.2.

Brain (1:1, w/v) or diaphragm or intercostal muscle homogenate (1:2, w/v), was incubated with Soman at a final concentration of 8 nM, for 10 min at 0° and pH 10. Drugs were then added separately. After incubation at 37° for 30 min, 0.08 N HCl was used to bring the pH back to neutral. Enzyme activity was estimated. The experimental data show (Table 2A) that these three drugs at final concentrations of 0.33 mM reactivated Soman-phosphonylated human tissue cholinesterase as effectively as the red cell enzyme. Experiments performed with rat tissues under the same conditions showed similar results (Table 2A).

Aged Soman-phosphonylated cholinesterase. Human red cells were inhibited with an equal volume of 40 nM Soman at 37°, pH 7.2, for 10 min and incubated with drugs separately at 26°, pH 7.2, for 30 min after washing. As shown in Table 2B, none of these drugs reactivated the aged Soman-phosphonylated red cell AChE.

Human brain, diaphragm and intercostal muscle homogenates were incubated with Soman at a final concentration of 8 nM for 10 min at 37° and pH 7.2. Drugs were then added separately. After incubation for 30 min, enzyme activity was assayed. Results showed that all three drugs at a final concentration of 0.33 mM failed to reactivate the aged Soman-phosphonylated human tissue cholinesterase (Table 2B). Experiments in rat tissues performed under the same conditions gave similar results (Table 2B).

The results show clearly that human and rat non-aged phosphonylated cholinesterases are reactivated by the three oximes tested in the presence of Sarin or Soman. HI-6 was the most potent reactivator. All of these drugs failed to reactivate the aged enzymes.

Table 2. Reactivation of Soman-phosphonylated human and rat cholinesterases

Tissue	Drug	Cholinesterase activity (%) ER	Cholinesterase activity (%) EI	Reactivation (%)
		(A)*		
Human				
Red cell	HI-6	49 ± 15	2 ± 2	67 ± 16
	HGG-42	40 ± 22	2 ± 2	31 ± 5
	2-PAM	55 ± 17	2 ± 2	22 ± 4
Brain	HI-6	70 ± 19	12 ± 6	65 ± 11
	HGG-42	51 ± 8	12 ± 6	61 ± 14
	2-PAM	102 ± 15	12 ± 6	25 ± 4
Intercostal muscle	HI-6	83 ± 6	6 ± 4	52 ± 14
	HGG-42	54 ± 7	6 ± 4	23 ± 4
Diaphragm	HI-6	87 ± 8	7 ± 8	55 ± 20
	HGG-42	74 ± 10	7 ± 8	24 ± 3
	2-PAM	90 ± 10	7 ± 8	21 ± 8
Rat				
Brain	HI-6	89 ± 17	10 ± 17	59 ± 4
	2-PAM	79 ± 22	10 ± 17	22 ± 12
Intercostal muscle	HI-6	69 ± 7	10 ± 3	70 ± 26
	2-PAM	68 ± 5	10 ± 3	40 ± 6
Diaphragm	HI-6	52 ± 11	8 ± 6	64 ± 21
	2-PAM	57 ± 7	8 ± 6	31 ± 11
		(B)†		
Human				
Red cell	HI-6	94 ± 5	9 ± 2	0
	HGG-42	88 ± 21	9 ± 2	0
Brain	2-PAM	79 ± 6	0 ± 1	0
Intercostal muscle	HGG-42	50 ± 13	1 ± 1	2 ± 3
	2-PAM	90 ± 3	1 ± 1	14 ± 7
Diaphragm	HI-6	61 ± 11	0	18 ± 5
	2-PAM	82 ± 19	0	9 ± 6
Rat				
Brain	HI-6	103 ± 1	5 ± 7	13 ± 1
	HGG-42	64 ± 11	5 ± 7	1 ± 1
	2-PAM	95 ± 23	5 ± 7	2 ± 1
Intercostal muscle	HI-6	84 ± 3	5 ± 4	18 ± 6
	HGG-42	62 ± 6	5 ± 4	0
	2-PAM	86 ± 13	5 ± 4	14 ± 5
Diaphragm	HI-6	87 ± 1	4 ± 5	19 ± 2
	2-PAM	81 ± 5	4 ± 5	9 ± 0

* (A) Human or rat ChEs; inhibition, 20 nM Soman for red cell, 8 nM for other tissues, 0°, pH 10, for 10 min; reactivation, 0.33 mM drugs, pH 10, 26° for red cell or 37° for other tissues, for 30 min; enzyme assay, 37°, pH 7.2, for 30 min; mean ± S.D., average of three determinations in duplicate. ER and EI are defined in Table 1.

† (B) Human or rat ChEs; inhibition, 20 nM Soman for red cell, 8 nM for other tissues, aged at 37°, pH 7.2, for 10 min; reactivation, 0.33 mM drugs, pH 7.2, 26° for red cell or 37° for other tissues, for 30 min; enzyme assay, 37°, pH 7.2, for 30 min; mean ± S.D., average of two determinations in duplicate.

Institute of Basic Medical Sciences
Beijing, China

MAN-CHI SUN*
FENG-ZHEN LI
TING-CHUNG CHOU

REFERENCES

1. O. L. Wolthuis and L. A. Kepner, *Eur. J. Pharmac.* **49**, 415 (1978).
2. O. L. Wolthuis, R. A. P. Vanwersch and H. J. Van der Wiel, *Eur. J. Pharmac.* **70**, 355 (1981).
3. J. T. Dodge, C. Mitchell and D. J. Hanahan. *Archs Biochem. Biophys.* **100**, 119 (1963).
4. S. Hestrin, *J. biol. Chem.* **180**, 249 (1949).
5. G. Gilbert, T. Wagner-Jauregg and G. M. Steinberg, *Archs Biochem. Biophys.* **93**, 469 (1961).
6. L. P. A. de Jong and G. Z. Worling, *Biochem. Pharmac.* **29**, 2379 (1980).
7. L. P. A. de Jong and G. Z. Worling, *Biochem. Pharmac.* **33**, 1119 (1984).

* To whom correspondence should be sent.

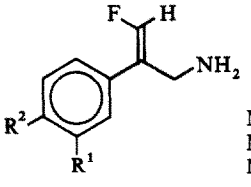
Biochemical Pharmacology, Vol. 35, No. 2, pp. 349–351, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

(*Z*)-2-(2,4-Dichlorophenoxy)methyl-3-fluoroallylamine (MDL 72638): a clorgyline analogue with surprising selectivity for monoamine oxidase type B

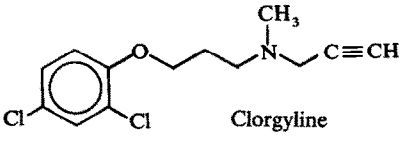
(*Received* 7 *June* 1985; *accepted* 5 *August* 1985)

We have recently reported [1–4] that derivatives of 2-aryl-3-fluoroallylamine and 2-arylalkyl-3-fluoroallylamine are potent enzyme-activated inhibitors of monoamine oxidase (MAO; EC 1.4.3.4). These substances generally show selectivity for the B-form of the enzyme. For example the 3,4-dimethoxyphenethylamine derivative MDL 72145 is approximately 100-fold selective in this respect. A few examples, notably the *meta*-tyramine derivative MDL 72392 and the dopamine analogue MDL 72394, nevertheless, did show modest selectivity (up to 10-fold) for the A-form of the enzyme. In general, the selectivity displayed by these inhibitors reflects the substance specificities of the corresponding phenethylamine derivatives [2].

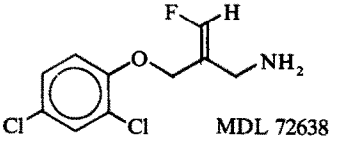


MDL 72145 $R^1 = R^2 = OCH_3$
MDL 72392 $R^1 = OH$; $R^2 = H$
MDL 72394 $R^1 = R^2 = OH$

Of the many studies aimed at developing an understanding of the structure–activity relationship regarding substrate and inhibitor specificities for one form of the enzyme or the other, the most accepted conclusion to date is that selectivity is related in part to the distance between the aromatic ring and the nitrogen atom [5, 6]. The type A selectivity of clorgyline has been attributed to the 4-atom linkage between the dichlorobenzene ring and the nitrogen atom. Since clorgyline is probably the most selective type A, irreversible inhibitor known, we decided to prepare a structurally related fluoroallylamine in an attempt to improve the modest A-selectivity seen in this series. Thus, the analogue MDL 72638 [(*Z*)-2-(2,4-dichlorophenoxy)methyl-3-fluoroallylamine] has been synthesized [7]. Although the exact conformation of clorgyline in the active site of MAO is not known, the conformational flexibility of both substances is similar so that, whatever the aromatic ring–nitrogen atom distance clorgyline adopts, MDL 72638 can more or less adopt the same. The average aromatic ring–nitrogen distance for the two molecules existing in a variety of conformations would appear to be very similar from an examination of Dreiding models.



Clorgyline



MDL 72638

We report in this communication that, despite this close structural relationship to clorgyline, MDL 72638 is a potent, very selective inhibitor of the B form of MAO.

Methods and results

When partially purified rat brain mitochondrial MAO was incubated [4] with varying concentrations of MDL 72638, time-dependent inhibition was observed. With either 5-hydroxytryptamine (5-HT; Type A) or phenethylamine (PEA; Type B) as substrate, pseudo first order kinetics were observed for at least three half-lives. The minimum half-life (τ_{50}) at saturating conditions and the apparent dissociation constant (K_i), calculated according to the method of Kitz and Wilson [8], were 8.9 min and 1.75 μM and 2.9 min and 0.088 μM for the A and B forms of MAO, respectively (Fig. 1 and Table 1). The selectivity for the B form of the enzyme, approximated from the ratio of concentrations of MDL 72638 required to inhibit both forms of the enzyme at the same rate, was found to be 100.

Discussion

The difference in MAO inhibitory selectivity between (*Z*) - 2 - (2,4 - dichlorphenoxy)methyl - 3 - fluoroallylamine (MDL 72638) and clorgyline is quite remarkable considering the close structural similarity between the two compounds. The K_i values of MDL 72638 for MAO A and B, respectively, are 30 times higher and 660 times lower than those of clorgyline [9]. Obviously, the relationship between the aromatic ring and nitrogen atom is in itself not a dominant feature in bestowing selectivity for one form of the enzyme or the other, at least when inhibitors from different structural classes of compounds are concerned. Within a particular series of inhibitors, however, this factor may still play a role. An explanation for the observed disparity in selectivity in the present case cannot be attempted with confidence until the mechanism of inactivation of MAO by MDL 72638 and related compounds has been fully elaborated. At first sight, there would appear to be three possible explanations. If both MDL 72638 and clorgyline were, after enzymic activation, to alkylate the same enzyme or cofactor group the selectivity could be attributed to a difference in the binding step to the two enzyme forms. This situation would be similar to that of clorgyline and L-deprenyl which are structurally related