Arh. hig. rada, *24* (1973) 199.

COMPARISON BETWEEN INHIBITION OF ACETYLCHOLINESTERASE AND CHOLINESTERASE BY SOME N-METHYL- AND NN-DIMETHYL-CARBAMATES

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(Recieved for publication September 12, 1973)

Second order rate constants for inhibition (k_a) of various sources of acetylcholinesterase (EC 3.1.1.7) and cholinesterase (EC 3.1.1.8) with seven N-methyl- and two NN-dimethylcarbamates are given in tabular form. A comparison between acetylcholinesterase and cholinesterase is presented for different stages of the time course of the reaction: at pre-steady state the comparison is based on k_a values, and at steady state on the K values (apparent equilibrium constants for enzyme and inhibitor). When two enzymes are inhibited by the same compound, the difference in the degree of inhibition at steady state and pre-steady state will be greater the greater the difference in the stability of the carbamylated enzymes.

Organophosphorus compounds and carbamates are inhibitors of acetylcholinesterase (E. C. 3.1.1.7) and cholinesterase (E. C. 3.1.1.8) because they acylate (phosphorylate and carbamylate) the active site of the enzyme. The degree of enzyme inhibition is defined by the rate constant of inhibition. However, when the inhibited enzyme is unstable and hydrolyses (reactivates spontaneously) to produce the active enzyme, the degree of inhibition also depends on the rate constant of spontaneous reactivation.

The over-all reaction between cholinesterases (E) and acylating inhibitors (I) is described by the following equation:

$$E + I \xrightarrow{k_a} EA \xrightarrow{k_r} E \qquad (1)$$

where EA is the inhibited (acylated) enzyme, k_a the second order rate constant of inhibition and k_r the first order rate constant of spontaneous reactivation.

When the time of the reaction between enzyme and inhibitor is short and the rate of spontaneous reactivation slow, the degree of inhibition depends primarily on the rate constant k_a. When inhibition of two enzymes by a given inhibitor is compared under the above conditions (pre-steady state inhibition) the enzyme with the higher k_a will be more inhibited. However, when the reaction proceeds for a longer time and the rate of inhibition gradually approaches that of spontaneous reactivation, a steady state inhibition is reached when these two rates become equal. At steady state, the concentration of the inhibited enzyme remains constant and the degree of inhibition is defined by the apparent affinity constant (K):

$$K = \frac{[EA]}{[E] \cdot [I]} = \frac{k_a}{k_r} \qquad (2)$$

This constant (or its reciprocal) was used earlier to define steady state inhibition (1—5).

When two enzymes are compared at steady state inhibition, it is the *K* constant which should be used for comparison. The enzyme with the larger *K* will be more inhibited at any given inhibitor concentration.

Extensive comparison has been done between acetylcholinesterase and cholinesterase with respect to inhibition by organophosphorus compounds (6—8).

Most phosphorylated cholinesterases are fairly stable (9) and the reaction with the enzyme never reaches a steady state. For the majority of organophosphorus compounds cholinesterase is inhibited more than acetylcholinesterase, e. i. k_a is larger for cholinesterase than for acetylcholinesterase. Such comparisons have not been done for carbamates.

In Table 1 are summarized some data for the inhibition of acetylcholinesterase and cholinesterase by several N-methylcarbamates and two N,N-dimethylcarbamates. Most of these compounds are used as insecticides. For a given compound, the k_a values for acetylcholinesterase from different sources are fairly similar; the same holds for inhibition of human and horse serum cholinesterase. For all compounds but one, acetylcholinesterase is inhibited more than cholinesterase; this is the reverse of what was shown for organophosphorus compounds. What correlation would hold for a wider range of carbamates, is unpredictable.

Carbamylated cholinesterases are unstable (cf. 9). The degree of inhibition therefore depends not only on the rate constant of inhibition (k_a), but also on the rate constant of spontaneous reactivation (k_r).

So it follows from Table 1 that the ratio of the rate constants of inhibition of human erythrocyte acetylcholinesterase and human plasma cholinesterase by 2-*iso*propoxyphenyl-N-methylcarbamate (OMS-33) is about 60. However at steady state serum cholinesterase will be inhibited by only a 16.5 times higher inhibitor concentration, if the same degree of inhibition of both erythrocyte and serum cholinesterase is to be obtained. This is due to a faster reactivation of monomethylcarbamylated erythrocyte cholinesterase the rate constants of reactivation for human

erythrocyte and human plasma being 0.0134 min^{-1} (*Simeon*, unpublished) and 0.0037 min^{-1} (10) respectively. The k_a ratio for human erythrocyte and human plasma cholinesterase inhibited by 3 methyl-5-*iso*propylphenyl-N-methylcarbamate (OMS-716) is only about 2 (Table 1) so that at steady state serum cholinesterase is even more inhibited, which is due to a slower rate of reactivation of the serum enzyme.

In Table 2. a theoretical example is given showing how the reactivation rate constants (k_r) affect the inhibition of the two enzymes at steady state. Enzymes I and II are either two different enzymes (acetylcholinesterase and cholinesterase) or the same enzyme from two different species (e. g. acetylcholinesterase from rat brain and fly head). It has been assumed that both enzymes have the same k_a for inhibition by all three compounds A, B and C (columns 1 and 2, Table 2). Consequently, at pre-steady state both enzymes will be equally inhibited by any of the three compounds. However, this will not necessarily be so at steady state. If the k_r constants are such as given in Table 2 (columns 3 and 4), the apparent equilibrium constants will be as stated in columns 5 and 6. It follows therefore that at steady state (last column, Table 2), compound A will be a better inhibitor of enzyme II than enzyme I, whereas compound C will be a better inhibitor of enzyme I than enzyme II; compound B will equally inhibit both enzymes at steady state like at pre-steady state.

The greater the difference between k_r values, the greater will be the difference between the degree of inhibition at pre-steady state and that at steady state. To know this difference is of interest when evaluating the insecticidal properties of anticholinesterases on one hand, and their toxic effects on the other (cf. 11). If steady state inhibition is established, the apparent equilibrium constant K is a better measure for predicting the degree of inhibition, than the rate constant k_a.

Table 1

Second order rate constants (k_a) for the inhibition of various preparations of acetylcholinesterase and cholinesterase by some carbamates.

The k_a values first reported in the present paper have been determined by the method described earlier (10). Inhibitors used as insecticides are also designated by their WHO code number. For commercial enzyme preparations the name of the firm (Sigma Chemical Co., St. Louis, Mo.; Winthrop Lab., New York, N. Y.; Worthington Biochemical Co., Freehold, N. Y.; A. B. Kabi, Stockholm) is given in brackets.

Inhibitor and enzyme	$10^{-4} \times k_a$ ($M^{-1}min^{-1}$)	Experimental conditions (° C, pH, buffer)	References
Phenyl-N-methylcarbamate (OMS-483)			
Acetylcholinesterase:			
— Bovine erythr. (Sigma)	0.026	25°, 7.0 5mM phosphate	Iverson and Main (12)
— Bovine erythr. (Winthrop)	0.027	25°, 7.4 100mM phosphate	Reiner and Simeon (4)
— Bovine erythr. (Sigma)	0.029	25°, 7.6 6mM phosphate	Hastings *et al.* (13)
— Bovine erythr. (Winthrop)	0.054	38°, 7.0 50mM phosphate	O'Brien *et al.* (14)
— Electric eel (Worthington)	0.037	25°, 7.0 5mM phosphate	Iverson and Main (12)
— Fly head	0.034	37.5, Warburg	Metcalf (15)
Cholinesterase:			
— Horse serum (Sigma)	0.009	25°, 7.4 100mM phosphate	This paper
2-isopropoxyphenyl-N-methylcarbamate (OMS-33)			
Acetylcholinesterase:			
— Bovine erythr. (Winthrop)	4.0	25°, 7.4 100mM phosphate	Reiner and Simenon (4)
— Bovine erythr. (Winthrop)	4.9	25°, 7.4 20mM phosphate	Reiner and Aldrige (16)
— Bovine erythr. (Winthrop)	10.6	38°, 7.0 50mM phosphate	This paper O'Brien *et al.* (14)
— Fly head	10.0	37.5 Warburg	Metcalf (15)
Cholinesterase:			
— Horse serum (Sigma)	0.36	25°, 7.4 100mM phosphate	Simeon *et al.* (10)
— Human serum (native)	0.095	25°, 7.4 0.15M NaCl	Reiner and Simeon (17)
— Dog plasma (native)	0.53	25°, 7.4 100mM phosphate	This paper

Inhibitor and enzyme	$10^{-4} \times k_a$ ($M^{-1}min^{-1}$)	Experimental conditions (° C, pH, buffer)	References
3-isopropylphenyl-N-methylcarbamate (OMS-162)			
Acetylcholinesterase:			
— Bovine erythr. (Sigma)	45.6	25°, 7.6 6mM phosphate	Hastings *et al.* (13)
— Bovine erythr. (Winthrop)	48.0	25°, 7.0 5mM phosphate	Iverson and Main (12)
— Bovine erythr. (Winthrop)	54.0	25°, 7.4 20mM phosphate	Reiner and Aldrige (16)
— Bovine erythr. (Winthrop)	54	25°, 7.4 100mM phosphate	Simeon (18)
— Electric eel (Worthington)	83.8	25°, 7.0 5mM phosphate	Iverson and Main (12)
— Fly head	25	37.5°	Metcalf (15)
Cholinesterase:			
— Horse serum (Sigma)	12.4	25°, 7.4 100mM phosphate	Simeon *et al.* (10)
— Human serum (AB Kabi)	8.0	25°, 7.4 20mM phosphate	Simeon *et al.* (10)
Monomethyl-neostigmine (Bromide of 3-trimethylaminophenyl N-methylcarbamate)			
Acetylcholinesterase:			
— Bovine erythr. (Winthrop)	2700	25°, 7.4 20mM phosphate	Reiner and Aldrige (16)
— Electric eel	900	25°, 7.0 100mM NaCl+ 20mM phosphate	Wilson *et al.* (2)
— Electric eel (Worthington)	2110	25°, 7.0 5mM phosphate	Iverson and Main (12)
Cholinesterase:			
— Horse serum (Sigma)	17.7	25°, 7.4 100mM phosphate	Simeon *et al.* (10)
— Human serum (AB Kabi)	26	25°, 7.4 100mM phosphate	Simeon *et al.* (10)
Naphthyl-N-methylcarbamate (OMS-29)			
Acetylcholinesterase:			
— Bovine erythr. (Winthrop)	1.8	25°, 7.4 100mM phosphate	Reiner and Simeon (4)
— Bovine erythr. (Sigma)	2.1	25°, 7.4 Na-barbital	Winteringham and Fowler (19)
— Bovine erythr. (Sigma)	2.18	25°, 7.6 6mM phosphate	Hastings *et al.* (13)
— Bovine erythr. (Winthrop)	12.5	38°, 7.0 50mM phosphate	O'Brien *et al.* (14)
— Fly head	74	30, 8.0 100mM phosphate	Kunkee and Zweig (3)
— Bee head	400	30, 8.0 100mM phosphate	Kunkee and Zweig (3)
— Fly head	7.7	37.5 Warburg	Metcalf (15)

Inhibitor and enzyme	$10^{-4} \times k_a$ ($M^{-1}min^{-1}$)	Experimental conditions (° C, pH, buffer)	References
Cholinesterase:			
— Horse serum (Sigma)	0.69	25°, 7.4 100mM phosphate	This paper
Eserine			
Acetylcholinesterase:			
— Bovine erythr. (Sigma)	325	25°, 7.6 6mM phosphate	Main and Hastings (20)
— Bovine erythr. (Sigma)	203	25°, 7.4 Na-barbital	Winteringham and Fowler (19)
— Electric eel	200	25°, 7.0 100mM NaCl+ 20mM phosphate	Wilson *et al.* (2)
— Spider mite	300	33°, 7.0 50mM pyro-phosphate 70mM NaCl	Smissaert *et al.* (21)
Cholinesterase:			
— Human plasma (native)	216	25°, 7.4 100mM phosphate	This paper
3-methyl-5-isopropylphenyl-N-methylcarbamate (OMS-716)			
Acetylcholinesterase:			
— Bovine erythr. (Winthrop)	32	25°, 7.4 100mM phosphate	This paper
— Human erythr. (native)	42.0	25°, 7.4 100mM phosphate	This paper
Cholinesterase:			
— Human serum (AB Kabi)	21	25°, 7.4 100mM phosphate	This paper
— Human plasma (native)	23.8	25°, 7.4 100mM phosphate	This paper
— Dog plasma (native)	41.9	25°, 7.4 100mM phosphate	This paper
Neostigmine (Methyl sulphate of 3-trimethylamino-phenyl NN-dimethylcarbamate)			
Acetylcholinesterase:			
— Bovine erythr. (Winthrop)	67	26°, 7.4 100mM phosphate	Simeon (18)
— Bovine erythr. (Winthrop)	244	25°, 7.4 20mM phosphate	Reiner and Aldrige (16)
— Bovine erythr. (Sigma)	400	25°, 7.0 5mM phosphate	Iverson and Main (12)
— Bovine erythr. (Sigma)	430	25°, 7.6 6mM phosphate	Hastings *et al.* (13)
— Electric eel (Worthington)	172	25°, 7.0 5mM phosphate	Iverson and Main (12)

Inhibitor and enzyme	$10^{-4} \times k_a$ ($M^{-1}min^{-1}$)	Experimental conditions (° C, pH, buffer)	References
— Electric eel	95	25°, 7.0 100mM NaCl+ 20mM phosphate	Wilson *et al.* (2)
Cholinesterase:			
— Horse serum (Sigma)	10.6	25°, 7.4 100mM phosphate	Simeon *et al.* (10)
— Human serum (AB Kabi)	16.7	25°, 7.4 20mM phosphate	Simeon *et al.* (10)
3-isopropylphenyl NN-dimethylcarbamate (OMS-476)			
Acetylcholinesterase:			
— Bovine erythr. (Winthrop)	0.29	25°, 7.4 20mM phosphate	Reiner and Aldridge (16)
Cholinesterase:			
— Human serum (AB Kabi)	0.90	25°, 7.4 20mM phosphate	Simeon *et al.* (10)

Table 2

Theoretical example (see text)

	Enz. I k_a	Enz. II k_a	Enz. I k_r	Enz. II k_r	Enz. I K	Enz. II K	K (Enz. I) : K (Enz. II)
Compound A	100	100	10	1	10	100	0.1
Compound B	100	100	10	10	10	10	1.0
Compound C	100	100	10	100	10	1	10.0

References

1. *Wilson, I. B., Hatch, M. A., Ginsburg, S.:* J. Biol. Chem., *235* (1960) 2312.
2. *Wilson, I. B., Harrison, M. A., Ginsburg, S.:* J. Biol. Chem., *236* (1961) 1498.
3. *Kunkee, R. E., Zweig, G.:* Biochem. Pharmacol., *14* (1965) 1001.
4. *Reiner, E., Simeon-Rudolf, V.:* Biochem. J., *98* (1966) 501.
5. *Kitz, R. J., Ginsburg, S., Wilson, I. B.:* Biochem. Pharmacol., *16* (1967) 2201.
6. *Hobbiger, F.:* Chem. Ind. (1954) 1574.
7. *Heath, D. F.:* Organophosphorus poisons, Pergamon Press, Oxford, London, New York, Paris, 1961, pp. 146 and 152.
8. *Koelle, G. B.* sub-editor: Cholinesterases and anticholinesterase agents; Eichler, O. and Farah, A. editors: Handbuch der Experimentallen Pharmakologie, Ergänzungswerk, Springer-Verlag, Berlin, Göttingen, Heidelberg, (1963) Vol. *15*, p. 449.
9. *Reiner, E.:* Bull. Wld Hlth Org., *44* (1971) 109.

10. *Simeon, V., Reiner, E., Vernon, C. A.:* Biochem. J., *130* (1972) 515.
11. *Aldridge, W. N.:* Bull. Wld Hlth Org., *44* (1971) 25.
12. *Iverson, F., Main, A. R.:* Biochemistry, *8* (1969) 1889.
13. *Hastings, F. L., Main, A. E., Iverson, F.:* J. Agr. Food Chem., *18* (1970) 497.
14. *O'Brien, R. D., Hilton, B. D., Gilmour, L.:* Mol. Pharmacol., *2* (1966) 593.
15. *Metcalf, R. L.:* Bull. Wld Hlth Org., *44* (1971) 43.
16. *Reiner, E., Aldridge, W. N.:* Biochem. J., *105* (1967) 171.
17. *Reiner, E., Simeon, V.:* Arch. Toxikol., *23* (1968) 237.
18. *Simeon, V.:* Comparative studies of the interaction of cholinesterase with inhibitors and substrates, Ph. D. thesis, University of Zagreb, Zagreb 1972.
19. *Winteringham, F. P. W., Fowler, K. S.:* Biochem. J., *101* (1966) 127.
20. *Main, A. R., Hastings, F. L.:* Science, *154* (1966) 400.
21. *Smissaert, H. R., Voerman, S., Oostenbrugge, L,- Renooy, N.:* J. Agr. Food Chem., *18* (1970) 66.

ACKNOWLEDGEMENT

This work was supported in part by a research grant PL 480 from the U.S.A. Environmental Protection Agency.

Sažetak

USPOREDBA IZMEĐU INHIBICIJE ACETILKOLINESTERAZE I KOLINESTERAZE NEKIM N-METALIMA I NN-DIMETILNIM KARBAMATIMA

U radu su dane konstante brzine inhibicije (k_a) acetilkolinesteraze (EC 3.1.1.1.7) i kolinesteraze (EC 3.1.1.1.7) različitih izvora, sa sedam N-metil- i dva NN-dimetilkarbamata. Acetilkolinesteraza i kolinesteraza uspoređene su u različitim stupnjevima toka inhibicije. Usporedba u predstacionarnom stanju bazirana je na k_a vrijednostima, a u stacionarnom stanju na K vrijednostima (prividna ravnotežna konstanta za enzim i inhibitor). Kada su dva enzima inhibirana istim spojem, razlika u stupnju inhibicije u predstacionarnom i stacionarnom stanju biti će veća što je veća razlika u stabilnosti karbamiliranih enzima.

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Primljeno 12. IX 1973.