Arh. hig. rada, 25(1974) 51.

EFFECT OF ACETYLCHOLINE ON INHIBITION OF CHOLINESTERASES BY ACYLATING INHIBITORS

VERA SIMEON

Institute for Medical Research, Yugoslav Academy of Sciences and Arts, Zagreb

(Received for publication February 8, 1974)

The effect of acetylcholine on inhibition of cholinesterases by acylating inhibitors is considered. The theoretical rates of inhibition in the absence and presence of acetylcholine are calculated. The K_m and K_{ss} constants used in calculation were both evaluated experimentally and taken from published data. It follows from the calculation that the reported concentration of acetylcholine in mammalian brain should provide protection to acetylcholinesterase against inhibition by organophosphates and carbamates.

It is known that substrates decrease the rate of acylation of cholinesterases by carbamates and organophosphates (cf. 1). This fact might be of importance in that the toxic effects caused by anticholinesterases *in vivo* could be decreased, if substrate concentrations are high enough to considerably slow down the enzyme inhibition.

Using published data for the concentrations of acetylcholine in mammals the effect of substrate on the rate of inhibition of cholinesterases was calculated. The calculations were based on kinetic equations derived from *in vitro* studies; it was anticipated that the concentrations of substrate and inhibitor were in excess over those of the enzyme.

In its simplest form the reaction between enzyme [E] and an acylating inhibitor [AB] can be presented by eqn. (1):

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

and between enzyme and substrate [S] by eqn. (2):

$$E + S \rightleftarrows ES \longrightarrow E + P \qquad (2)$$

$\langle EA \rangle$ stands for the acylated enzyme, which is either the phosphorylated or carbamylated cholinesterase; k_a is the second order rate constant of inhibition. $\langle ES \rangle$ is the Michaelis complex between substrate and enzyme and $\langle P \rangle$ are the products of substrate hydrolysis.

At a given inhibitor concentration the rate of reaction (1) is determined by the first order rate constat $\langle K \rangle$ which is:

$$k = k_a [AB] \tag{3}$$

where [AB] is the molar concentration of the inhibitor. When reaction (1) proceeds in the presence of substrate the first order rate constant of inhibition (k') will be (cf. 1):

$$k' = \frac{k_a [AB]}{1 + [S]/K_m} \tag{4}$$

K_m is the Michaelis constant for reaction (2).

For acetylcholinesterase acetylcholine is not only a substrate but also an inhibitor. The mechanism of the reaction in excess of substrate can be very complex (1). However, in its simplest form the reaction will be:

$$ES + S \rightleftarrows ESS \tag{5}$$

In such case the first order rate constant of inhibition in the presence of substrate becomes (cf. 1, 2):

$$k' = \frac{k_a [AB]}{1 + [S]/K_m + [S]/K_m \cdot K_{ss}} \tag{6}$$

K_{ss} is the dissociation constant for the ESS complex in eqn. (5).

The equations (1), (2) and (5) present the reaction between enzyme, substrate and inhibitor in its simplest form i. e. substrate and inhibitor bind to the same site of the enzyme and the concentration of the Michaelis type complex between enzyme and inhibitor is negligible. Eqns. (4) and (6) were derived on the above assumptions (cf. 1, 2).

Knowing K_m and K_{ss} it is possible to calculate the relationship between the rate of inhibition in the presence and absence of substrate.

The published K_m values for acetylcholinesterase and acetylcholine cover a wide range from 0.05 to 0.64 mM (cf. 3). The experimental conditions also vary, particularly the substrate concentrations wherefrom the K_m values were calculated. Taking the view that K_m should be obtained from experiments where $0.1\ K_m \leq [S] \leq 10\ K_m$ only data (3—6) that fulfilled this requirement were taken into the calculations. The K_m

values for acetylcholinesterase from human and bovine erythrocytes selected in that way are in the range from 0.06 to 0.21 mM. Our own K_m values for acetylcholinesterase from human erythrocyte is (0.06 ± 0.01) mM (determined by the pH-stat method [4] at 37°C with 0.032 to 1.0 mM acetylcholine). The mean value of the K_m from literature data (3—6) and our own is 0.14 mM.

The published K_{ss} constants for acetylcholine and human and bovine erythrocyte acetylcholinesterase range from 11 to 35 mM (cf. 3). Including our own K_{SS} value of 16 mM (determined on human erythrocytes at 37°C) the mean value for the K_{ss} constant is 18 mM.

The published K_m values for human and horse serum cholinesterase are from 1.2 to 3.2 mM (cf. 3). The K_m value for human plasma determined by the above mentioned method (4) is (2.3 ± 0.5) mM (the range of substrate concentration was 0.032 to 100 mM). The mean value of the K_m for serum cholinesterase and acetylcholine from literature data and our own is therefore 1.8 mM.

Taking the mean K_m and K_{ss} values, the influence of acetylcholine on the first order rate constant of inhibition (k') was calculated (Table 1). For acetylcholinesterase eqn. (6) was used and for cholinesterase eqn. (4). In both cases the expression k_a [AB] was arbitrarily set as 100 so that the results in Table 1 apply to any inhibitor and any inhibitor concentration for which inhibition follows eqn. (1).

It follows from Table 1 that when the substrate concentration is 10 μM or less the rate of inhibition is almost the same as in the absence of substrate (Table 1). Substrate concentrations above 10 μM will decrease the rate of inhibition and this effect is more pronounced the higher the substrate concentration. A given substrate concentration will

Table 1

The effect of acetylcholine on the inhibition of acetylcholinesterase and cholinesterase by anticholinesterases. k' is the first order rate constant of inhibition in the presence of substrate calculated by eqn. (6) for acetylcholinesterase and by eqn. (4) for cholinesterase. The expression $k_a \langle AB \rangle$ is arbitrarily set as 100

Acetylcholine concentration	Acetylcholinesterase K_m=0.14mM, K_{ss}=18mM	Cholinesterase K_m=1.8 mM
0	100	100
1 μM	99.3	99.9
10 μM	93.3	99.5
0.1 mM	58.2	94.7
1 mM	11.7	64.3
10 mM	0.9	15.3

protect more efficiently erythrocyte than serum cholinesterase. This is due to two reasons: (a) the K_m for acetylcholinesterase is smaller than for serum cholinesterase and (b) the formation of ESS complex provides an additional protection against the inhibitor. It follows further from Table 1 that protection of serum cholinesterase becomes appreciable when acetylcholine concentration is higher than 0.1 mM, while at that concentration the protection of acetylcholinesterase is very pronounced, i. e. the rate of inhibition is roughly half of that in the absence of substrate.

It is difficult to predict the *in vivo* effects of acetylcholine, because the concentrations *in situ* of both enzyme and substrate are not known. Nevertheless, summarizing the published data on acetylcholine contents *in vivo* and assuming an even distribution of substrate in tissue, some assessments can be made. In Table 2 are presented the concentrations

Table 2

The concentrations of acetylcholine in different preparations calculated from published data on acetylcholine content assuming an even distribution. In brackets are given the inhibitors in the presence of which the acetylcholine content was determined

Preparation	Acetylcholine (μM)	References
Man		
Plasma	0.07—0.22	7
Brain	0.6 —4.1	8
Rat		
Brain	14	9
Brain	20—40	10
Brain	26	11
Brain (phosdrine)	30	9
Brain (eserine)	34	12
Brain (DFP)	34	13
Blood (paraoxon)	0.2	4
Mouse		
Brain	13	14
Hypothalamus	23	14
Dog		
Blood (paraoxon)	0.2	4
Cat		
Blood (paraoxon)	0.2	4

of acetylcholine calculated according to the above mentioned assumption. The concentration of acetylcholine in blood is about 0.2 μM and this concentration, evidently, can provide no protection against an inhibitor (cf. Table 1). The concentrations of acetylcholine in mammalian brain appear to be 10 μM or higher. Such concentrations should provide some protection against an inhibitor, particularly if acetylcholine is not equally distributed in which case it could be very efficient in competing with an inhibitor for the active site of acetylcholinesterase. In case of anti-cholinesterase poisoning the accumulated acetylcholine will decrease the rate of inhibition thus providing more time for the inhibitor to become removed from its place of action and for the enzyme to become spontaneously reactivated.

ACKNOWLEDGEMENT

The author wishes to thank Dr. E. Reiner for her valuable suggestions and discussion. This work was supported in part by research grants from the World Health Organisation, Geneva and PL 480 grant from U.S. Environmental Protection Agency.

References

1. *Aldridge, W. N., Reiner, E.:* Enzyme Inhibitors as Substrates, Interaction of esterases with esters of organophosphorus and carbamic acids, North Holland Pub. Co., Amsterdam, London, 1972, pp. 268—275.
2. *Reiner, E.:* personal communication.
3. *Simeon, V.:* Arh. hig. rada, *23* (1972) 29.
4. *Jensen-Holm, J.:* Acta pharmacol. et toxicol., *18* (1961) 379.
5. *Winteringham, F. P. W., Fowler, K. S.:* Biochem. J., *115* (1969) 147.
6. *Wright, C. I., Sabine, J. C.:* J. Pharm. Exp. Therap., *93* (1948) 230.
7. *Scudomore, H. H., Vorhaus, L. J., Kark, R. M.:* J. Lab. Clin. Med., *37* (1951) 860.
8. *Barsoum, G. S.:* J. Physiol., *84* (1935) 259, cit. by Biochemists' Handbook, ed. C. Long, Spon. Ltd. London, 1961, p. 648.
9. *Stavinoha, W. B., Ryan, L. C.:* Pharmacol. Exp. Therap., *150* (1965) 231.
10. *Hanin, I., Massarelli, R., Costa, E.:* Science, *170* (1970) 341.
11. *Szilagyi, P. I. A., Green, P. J., Monroe Brown, O., Margolis, S.:* J. Neurochem., *19* (1972) 2555.
12. *Slater, P.:* Arch. Int. Pharmacodyn., *181* (1969) 253.
13. *Fonnum, F., Guttormsen, D. M.:* Experientia, *25* (1969) 505.
14. *Feigenson, M. E., Saelens, J. K.:* Biochem. Pharmacol., *18* (1969) 1479.

Sažetak

UTJECAJ ACETILKOLINA NA INHIBICIJU KOLINESTERAZA ACILIRAJUĆIM INHIBITORIMA

Razmotren je utjecaj acetilkolina na inhibiciju kolinesteraza acilirajućim inhibitorima. Izračunata je teoretska brzina inhibicije u prisutnosti i odsutnosti acetilkolina. Za račun teoretske brzine inhibicije uzete su K_m i K_{ss} konstante dobivene u vlastitim pokusima i iz literature. S obzirom na poznate koncentracije acetilkolina u mozgu sisavaca, zaključeno je da bi taj supstrat trebao u nekoj mjeri zaštititi acetilkolinesterazu od inhibicije organofosfatima i karbamatima.

Institut za medicinska istraživanja i medicinu rada JAZU, Zagreb

Primljeno 8. II 1974.