KINETICS OF HYDROLYSIS OF THIOCHOLINE ESTERS BY PLASMA CHOLINESTERASES FROM VARIOUS ANIMALS

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The enzymatic characteristics of plasma cholinesterases have been studied by various authors[1-10] the views on some of their properties, however, are not uniform. It is not merely the matter of the action of activators or inhibitors[2-4,6-8] of these enzymes but also of the size of their K_m and V_{max} constants[3-5,9,10]. The hydrolysis of thiocholine esters has been studied so far only with human and horse plasma[3,4,9,10]. Since thiocholine esters have been used still more extensively as substrates for the determination of the activity of cholinesterases it has become necessary to study the kinetics of their enzymatic hydrolysis by plasma cholinesterases from various species.

EXPERIMENTAL

The activity of the cholinesterases was measured by our own modification[11] of the method of Szasz[3], using 5,5'-dithio-bis(2-nitrobenzoic) acid (Serva, Entwicklungslabor, Heidelberg) as chromogen, and acetylthiocholine and butyrylthiocholine iodides (Lachema, Brno) as substrates. The reaction was allowed to proceed in 0·2M Tris-HCl buffer, pH 7·6, at 25°C. The change in absorbance was measured at 412 nm (Vitatron, Sci. Instr., Holland). The rate of hydrolysis was expressed as μmol of substrate hydrolyzed by 10 μl of plasma in 1 min. Cysteine (Koch-Light Lab.) was used to obtain the calibration graph. Plasma of horse, guinea pig, rat, pig, mouse, and man served as sources of the enzyme. The number of individuals from which the plasma was obtained was at least 3 and 10 at the most. The constants for each individual sample of plasma were determined independently. The number of measurements of the constant varied between 10 and 24. The concentration range of the substrate was $1 \cdot 10^{-2} - 1 \cdot 10^{-4}$M. The constants were calculated[12] in Minsk 22 computer.

RESULTS AND DISCUSSION

The rate of hydrolysis of the substrates studied is pH-dependent. According to the reported data, however, spontaneous hydrolysis of acetylthiocholine and butyrylthiocholine[3] also increases with increasing pH. For the determination of K_m and V_{max}, pH 7·6 was chosen since at this value all the plasma cholinesterases examined in this study show at least 90% of their activity and spontaneous hydrolysis is negligible.

The computer program permitted three K_m- and V_{max}-values together with their corresponding confidence limits to be obtained from three transformations ($A - 1/[S]$ *versus* $1/v$, $B - v/[S]$ *versus* v, and $C - [S]/v$ *versus* $[S]$) (ref.[13,14]). An example of the enzymatic hydrolysis of butyrylthiocholine is given in Fig. 1. The nonlinear profile of the Lineweaver-Burk plot[13] leads to the conclusion[5,15-17] that two or more enzymes participate on the hydrolysis of this substrate. The same reaction of acetylthiocholine as substrate is shown in Fig. 2. The size of individual K_m and V_{max}-values as obtained by the most common, *i.e.* Lineweaver-Burk plot is given in Table I.

The results obtained in this study indicate that the affinity of plasma cholinesterases of the investigated species is higher for butyrylthiocholine than for acetylthiocholine. Similar observations have been made also with butyrylcholine[2]. Choline esters of butyric acid are regarded as more specific substrates of plasma cholinesterases[2,7]. A certain exception represents rat plasma cholinesterase which has been considered to be a propionylcholinesterase[2,18]. Our results (the relatively high K_m-value for one considered enzyme) also point to the exceptional features of cholinesterase of this species. The affinity of plasma cholinesterases for thiocholine esters is higher than its affinity for choline esters[7] recorded in the literature.

A comparison of the dispersion of the values of individual constants showed that the dispersion was smallest, both for K_m and also for V_{max}, when the values were as given under *B*. The size of the constants was slightly different when plot *A* or *C* were employed.

The programs used here permit also the calculation of Hill's coefficient *n*, *i.e.* of the number of binding sites in the molecule of cholinesterase which bind the given substrate. This fact together with the almost identical values of pH-optimums of all the plasma preparations investigated is indicative of the identical character of the active site of plasma cholinesterases of various species.

An important role in the exact determination of the two constants plays also the employed concentration range of the substrate. This range must be broad enough for all possible abnormalities in the corresponding transformations to become obvious. Our results can be compared with the data of Szasz[3] who determined K_m for human blood plasma cholinesterase as being $1{\cdot}2 \cdot 10^{-4}$M for acetylthiocholine as substrate and $6{\cdot}5 \cdot 10^{-5}$M for butyrylthiocholine as substrate. He did not observed a nonlinearity in the Lineweaver-Burk plot. Humiston and Wright[4] examined the enzymatic hydrolysis of acetylthiocholine by human plasma cholinesterase and concluded from the nonlinear Lineweaver-Burk plot that two enzymes with K_m-values $1{\cdot}5 \cdot 10^{-4}$M and $3{\cdot}5 \cdot 10^{-4}$M were present. The authors used different concentration ranges of the substrate. Our results evidence the presence of at least two forms of cholinesterase in human plasma, both for acetylthiocholine and butyrylthiocholine as substrate. Cholinesterase isozymes whose presence was evidenced by the nonlinear profile of the Lineweaver-Burk plot with both substrates, were

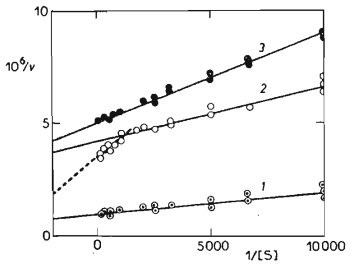

FIG. 1

Lineweaver-Burk Plot of Butyrylthiocholine Hydrolysis by Plasma Cholinesterases

1 Horse, *2* guinea pig, *3* rat.

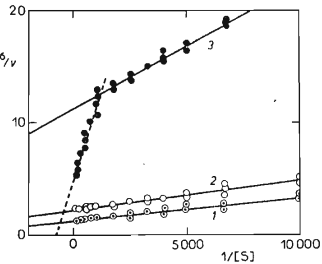

FIG. 2

Lineweaver-Burk Plot of Acetylthiocholine Hydrolysis by Plasma Cholinesterases

Designation the same as in Fig. 1.

TABLE I

K_m- and V_{max}-Values of Hydrolysis of Acetylthiocholine and Butyrylthiocholine by Plasma Cholinesterases, Obtained by Lineweaver-Burk Plot

The K_m-values are given in mM, the V_{max}-values in μmol/min of substrate hydrolyzed by 10 μl of plasma.

Source of plasma	Acetylthiocholine		Butyrylthiocholine	
	K_m	V_{max}	K_m	V_{max}
Horse	0·17	0·78	0·10	1·13
Man[a]	0·35	0·50	0·25	0·87
	0·06	0·46	0·06	0·77
Pig[a]	0·65	0·11	0·71	0·11
	0·12	0·08	0·22	0·08
Mouse[a]	0·47	0·44	0·34	0·62
	0·09	0·36	0·10	0·46
Guinea pig[b]	0·12	0·49	0·24	0·29
	—	—	0·06	0·24
Rat[c]	1·31	0·21	0·08	0·20
	0·10	0·09	—	—

[a] The profile of the Lineweaver-Burk plot is nonlinear for both substrates used. [b] The profile of the Lineweaver-Burk plot is nonlinear for butyrylthiocholine.[c] The profile of the Lineweaver-Burk plot is nonlinear for acetylthiocholine.

also observed in pig and mouse plasma. In rat plasma, at least two isozymes hydrolyzing acetylthiocholine and only one isozyme hydrolyzing butyrylthiocholine were observed. The opposite holds true for guinea pig plasma. Several molecular forms of plasma cholinesterase of various species were revealed predominantly by separation techniques[8,19–22]. Conclusions on the presence of isozymes made on the basis of the knowledge of the substrate hydrolysis require that the isozymes be present in adequate quantities, as has been shown for the isozymes rat liver esterases[23]. A condition of good reproducibility of the results is the measurement of the rate of the enzymatic hydrolysis of the substrate in the possibly broadest concentration range. The violation of these rules is the possible explanation of certain discrepancies in recorded data[3,4,9] on the K_m-value of the some enzyme.

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REFERENCES

1. Goedde H. W., Doenicke A., Altland A.: *Pseudocholinesterasen.* Springer Verlag, Heidelberg, New York 1967.
2. Svensmark O.: Acta Physiol. Scand., Suppl. *245*, 1965.
3. Szasz G.: Clin. Chim. Acta *19*, 191 (1968).
4. Humiston L. G., Wright G. J.: Toxicol. Appl. Pharmacol. *10*, 467 (1967).
5. Christian S. T., Beasley J. G.: J. Pharm. Sci. *57*, 1025 (1968).

6. Castro J. A.: Biochem. Pharmacol. *17*, 295 (1968).
7. Golikov S. N., Rozengart V. I.: *Cholinesterazy i Anticholinesteraznyje Veščestva*, p. 27. Medicina, Leningrad 1964.
8. Bajgar J.: Suppl. Sborníku věd. prací lékařské fakulty, Hradec Králové *10*, 509 (1967).
9. Berry W. K.: Biochim. Biophys. Acta *39*, 346 (1960).
10. Ioriom M. A., Michalek H., Chiavarelli S.: Mol. Pharmacol. *5*, 580 (1969).
11. Bajgar J., Patočka J.: Sborník věd. prací Vojenského lékařského výzkumného a doškolovacího ústavu, Hradec Králové *44*, 3 (1969).
12. Patočka J., Tulach J.: *Sborník abstrakt, 5. celostátní biochemický sjezd, Brno 1969*, p. 38.
13. Lineweaver H., Burk D.: J. Am. Chem. Soc. *56*, 658 (1934).
14. Augustinsson K. B.: Acta Physiol. Scand., Suppl. *52*, 1948.
15. Reiner J. M.: *Behavior of Enzyme Systems*, Burgess Publ. Co., p. 102, Minneapolis 1959.
16. Walter C.: *Steady State Applications in Enzyme Kinetics*, The Ronald Press Co., p. 89. New York 1964.
17. Dixon M., Webb E. C.: *Enzymes*, p. 89. Academic Press, New York.
18. Augustinsson K. B.: Acta Chem. Scand. *13*, 1097 (1959).
19. Juul P.: Clin. Chim. Acta *19*, 205 (1968).
20. Hess A. R., Angel R. W., Barron K. D., Bernsohn J.: Clin. Chim. Acta *8*, 656 (1963).
21. Oki Y., Oliver W. T., Funnel H. S.: Nature *8*, 605 (1964).
22. Dubbs C. A., Vivonia C., Hilburn J. M.: Science *131*, 1525 (1960).
23. Bajgar J., Patočka J.: Suppl. Sborníku věd. prací lékařské fakulty Hradec Králové *12*, 333 (1969).

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