SOME ENZYMATIC PROPERTIES OF HUMAN BRAIN ACETYLCHOLINESTERASE

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Received 19 December 1968

1. Introduction

The esterases of human brain are discussed in several papers [1–7], which deal with the study of isoenzymes of non-specific esterases. Most authors used the electrophoresis in starch gel for their separation [1,3–7]. This study describes only soluble esterases. Only some of these papers deal with soluble acetylcholinesterase (EC 3.1.1.7), too.

It is known that the human brain contains three isoenzymes of ACHE, which have different electrophoretic mobilities in starch gel [3,4,7].

Two of these isoenzymes could be characterized by measurement of their K_m value and I_{50} for some inhibitors. Their enzymatic properties were practically similar. The Michaelis constants of ACHE in human brain extract were 2.2×10^{-4} M and for both isoenzymes 1.5×10^{-4} M and 1.3×10^{-4} M, respectively, for ACH as substrate.

In this communication the existence of isoenzymes of ACHE with different enzymatic properties is discussed.

2. Materials and methods

The human brain was obtained from autopsy material of two men (45 and 55 years). Causa mortis: infarctus myocardii. Material was taken 24 hours after death. The brain was frozen (–35°) and part of the caput nuclei caudati (weight about 5 g) was taken for experimental purposes. The sample of brain tissue was homogenized (Ultra-Turrax, Germany) in three volumes of saline. The homogenate was divided into small tubes which were stored at –35° in the refrigerator.

The electrometric method with direct registration [8] was used for enzymatic activity measurements. All experiments were made at 25° and pH 8.0. The Michaelis constants were obtained by Lineweaver-Burk's method and the programme [8] for the computer MINSK 22 was used for their calculation. As substrate were used ACH, BuCH, and ATCH (Lachema, Brno). Used detergents Tween 20 and 80 were obtained from Koch-Light Laboratories.

Abbreviations: ACHE, acetylcholinesterase; ACH, acetylcholine; ATCH, acetylthiocholine, BuCH, butyrylcholine.

3. Results and discussion

3.1. *The influence of detergents Tween 20 and 80 on the velocity of hydrolysis of ACH by human brain ACHE*

It is known that some non-ionic surface active compounds (Tweens, Triton X-100, etc.) release the ACHE activity from cell structures, probably by destroying the lipoprotein complex.

Our experiments showed that the total activity in the presence of 1% of Tween 20 and 80 is diminished. Tween 20 reduces the total activity to 72% and Tween 80 to 58%. In further experiments the modification without Tweens was used.

3.2. *Hydrolysis of ACH, BuCH, and ATCH by human brain ACHE*

We found repeatedly that the curve velocity-concentration for ACH had two peaks (fig. 1). This is probably due to the presence of two or more iso-

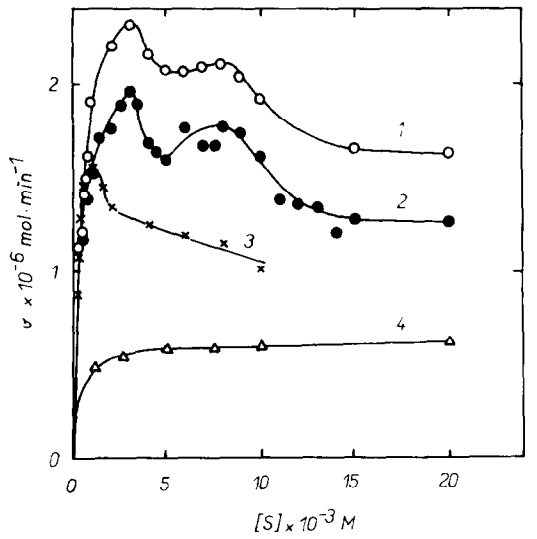

Fig. 1. The velocity of hydrolysis of some acetylcholine substrates by human brain ACHE. 1 and 2 – acetylcholine, 3 – acetylthiocholine, 4 – butyrylcholine.

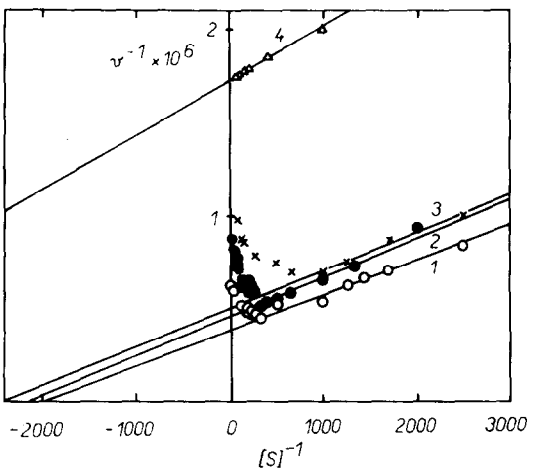

Fig. 2. Lineweaver-Burk's plotting of data from fig. 1. Michaelis constants for acetylcholine (1 and 2), acetylthiocholine (3), and butyrylcholine (4).

enzymes of ACHE with different affinity to ACH. The results obtained in experiments with BuCH as substrate showed that this second substrate optimum is not caused by the presence of non-specific cholinesterase (EC 3.1.1.8). The hydrolysis of ATCH by brain homogenate has only one substrate optimum (fig. 1).

3.3. K_m *values of human brain ACHE for ACH, BuCH and ATCH as substrates*

The graphic method of Lineweaver-Burk [9] was used for measurement of the Michaelis constants (fig. 2). The results are included in table 1.

Table 1

K_m and V_m constants of human brain ACHE for some acetylcholine substrates.

Substrate	K_m (M)	V_m (mol·min^{-1}·60mg^{-1})
Acetylcholine	$4.42 \pm 0.23 \times 10^{-4}$	$2.20 \pm 0.06 \times 10^{-6}$
Acetylcholine	$4.30 \pm 0.30 \times 10^{-4}$	$2.55 \pm 0.19 \times 10^{-6}$
Acetylthiocholine	$4.16 \pm 0.29 \times 10^{-4}$	$1.98 \pm 0.11 \times 10^{-6}$
Butyrylcholine	$1.72 \pm 0.18 \times 10^{-4}$	$0.58 \pm 0.07 \times 10^{-6}$

The Michaelis constants for ACH could be calculated only for velocities measured at low substrate concentrations. The obtained values of K_m were found to be identical for both brains. Bernsohn et al. [4] obtained values of a half of those given in this paper. The Michaelis constant for ACH is practically identical as for ATCH. The value of K_m for BuCH is different from these two values.

Acknowledgement

The authors thank Mr. Oleg Ochrymovič for technical assistance.

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