Aluminium Activation and Inhibition of Human Brain Acetylcholinesterase *in vitro*

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Abstract

The kinetic of interaction of human brain *caudate nucleus* acetylcholinesterase by Al^{3+} was studied, and the V_{max} and K_m parameters for the natural substrate acetylcholine were estimated as a function of Al^{3+} concentration by a titrimetric method. Noncompetitive activation for low Al^{3+} concentrations and noncompetitive inhibition (K_i = 1.56 mM) for higher Al^{3+} concentrations were observed. The activation as well as the inhibition of acetylcholinesterase by Al^{3+} is entirely allosteric, and Al^{3+} binds to the so-called β-anionic site of the enzyme. The results indicate a slightly positive cooperative effect of binding of Al^{3+} to acetylcholinesterase (Hill coefficient 1.34).

Introduction

Elevated brain Al^{3+} levels were observed in patients with loss of memory and a form of dementia often compared to the senile dementia of the Alzheimer type [1–3]. Since recent studies indicated that in Alzheimer's disease there is a disturbance in the cholinergic system of the brain [4–6], the influence of aluminium on the activity of the cholinergic enzyme acetylcholinesterase (EC 3.1.1.7, AChE) was studied [7–9]. Bovine erythrocyte [7], electric organ of *Electrophorus electricus* [8] and bovine brain [9] were the enzyme sources in these experiments. It was found that aluminium in low concentrations elevated the activity of AChE; on the other hand, higher concentrations produced inhibition of the enzyme activity [7, 9].

The observation of *in vitro* influence of aluminium on the AChE extracted from human brain *caudate nucleus* is the aim of this study.

Experimental

Human brains samples were obtained from autopsy material (Department of Forensic Medicine, Medical Faculty of Charles University, Hradec Králové, Czechoslovakia) from two individuals deceased after heart failure. A part of the *caput nuclei caudati* from each of the brains was homogenized with an Ultra-Turrax (F.R.G.) in five volumes of saline in the presence of 0.5% (*w*/*v*) Triton X-100 (Koch-Light). The homogenate was centrifuged 1 h at 105 000 g in a Super-speed 50 ultracentrifuge (MSE) and the supernatant was divided into small tubes and stored at –20 °C.

Atropine sulphate was purchased from Lékařské zásobování, Prague, and 9-amino-1,2,3,4-tetrahydroacridine hydrochloride (tacrine) was prepared by Dr. J. Bielavský as described previously [10]. $KAl(SO_4)_2 \cdot 12H_2O$, $AlCl_3 \cdot 6H_2O$ and $Al_2(SO_4)_3$ were used for the experiments. Stock Al^{3+} solutions were prepared fresh daily in distilled water. Acetylcholine iodide and all other commonly used chemicals were obtained from Lachema, Brno. Chemicals were of analytical grade and were used without further purification.

The activity of AChE was measured titrimetrically [11] as the initial rate of substrate hydrolysis with a pH-stat radiometer with acetylcholine iodide as a substrate. The acetic acid released was titrated with 0.05 N NaOH and the measurement was carried out in 0.15 M NaCl at pH 8.0 and 25 °C. A correction for spontaneous hydrolysis of the substrate was always applied.

All experiments with aluminium and other compounds used as second ligands were carried out in the same medium. The kinetic of AChE activation and inhibition was graphically evaluated according to Hanes [12], Hill [13], and Yonetani and Theorell [14]. The best fit to the experimental data points was calculated by linear regression analysis in a Hewlett-Packard 9830 A computer.

Results

The effects of aluminium concentration on human *caudate nucleus* AChE activity are shown in Fig. 1a. Low concentrations of aluminium (10–200 μM) caused an increase of the AChE activity; higher

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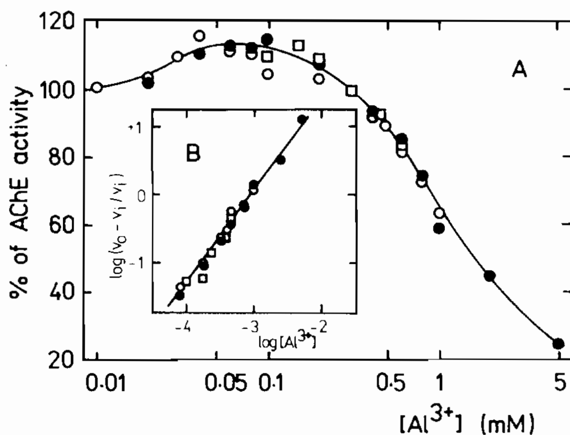

Fig. 1. (a) The effect of aluminium concentration on the activity of human brain acetylcholinesterase. (b) Replot of the $\log(v_0 - v_i/v_i)$ against the log $[Al^{3+}]$, *i.e.*, the Hill plot, was applied to high concentrations of aluminium. ○ $KAl(SO_4)_2$, ● $Al_2(SO_4)_3$, □ $AlCl_3$.

concentrations (400 to 5000 μM) produced the inhibition of the enzyme activity. The maximum activation was observed at 50 to 100 μM Al^{3+}. The concentration of Al^{3+} which diminished the activity of AChE by one-half (I_{50}) was 1.7 mM. The Hill coefficient (n) was 1.34 as determined from the slope of Fig. 1b.

A non competitive activation of aluminium at low concentrations and a noncompetitive inhibition at higher concentrations was observed by the Hanes plot (Fig. 2). A value of inhibition constant K_i of 1.56 mM was calculated.

In order to obtain more information about the binding site of Al^{3+} on AChE, experiments with a second inhibitor ligand with a known binding site were performed. Atropine which binds to the peripheral anionic site [15] also called β-anionic site [16] and tacrine which binds to the hydrophobic area of the active surface of AChE [17, 18] were used, respectively, as second ligands.

The combined inhibitory effects of aluminium and atropine on the activity of AChE in the Yonetani–Theorell plot of $1/v$ *versus* atropine concentration at varying concentrations of the aluminium are shown in Fig. 3. The series of parallel lines indicates a mutual competition of both inhibitors for the same site of the active surface of the AChE. Similar data for the combined inhibitory effects of aluminium and tacrine are given in Fig. 4. The Yonetani–Theorell plot shows a series of lines intersecting at one point, indicating that each of the two inhibitors binds to a different site of the active surface of AChE. The calculated value of the interaction constant, $\alpha = 0.2$, indicates that there is positive interaction ($\alpha < 1$) between aluminium and tacrine; hence the binding

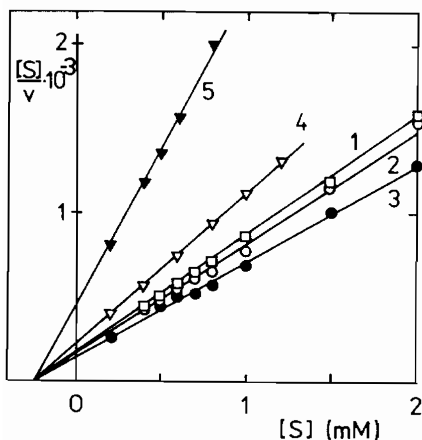

Fig. 2. Hanes plot demonstrating the effect of aluminium on the rate of hydrolysis of acetylcholine by human brain acetylcholinesterase. (1) no aluminium; (2) 0.06; (3) 0.04; (4) 0.5; (5) 2.0 mM Al^{3+} salt.

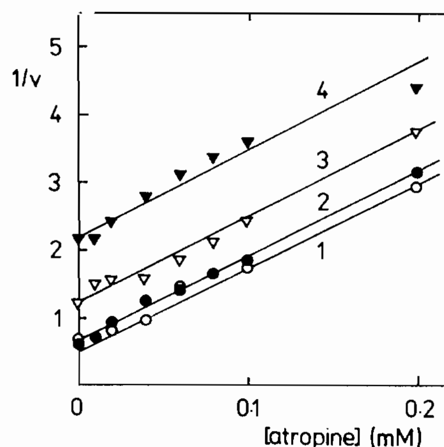

Fig. 3. The effect of aluminium on human brain acetylcholinesterase inhibition by atropine in Yonetani–Theorell plot. (1) no aluminium; (2) 0.04; (3) 1.0; (4) 2.0 mM Al^{3+} salt.

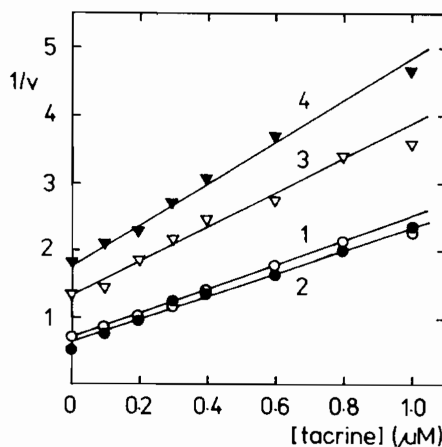

Fig. 4. The effect of aluminium on human brain acetylcholinesterase inhibition by tacrine in Yonetani–Theorell plot. (1) no aluminium; (2) 0.04; (3) 1.0; (4) 2.0 mM Al^{3+} salt.

of one inhibitor to the enzyme enhances the affinity of the binding of the second inhibitor.

Discussion

The activation effect of low aluminium concentrations on the AChE activity as well as the inhibition by higher aluminium concentration were observed by others [7, 9, 19]. No differences were observed by changing the type of aluminium salt, *i.e.*, $AlCl_3$, $Al_2(SO_4)_3$ and $KAl(SO_4)_2$. All experiments were carried out in high ionic strength media (0.15 M NaCl), because in this case the activation effect is higher [7, 9]. Presumably this is not an effect of the ionic strength but a specific effect of Na^+, due to a possible binding to the anionic subsite of the catalytic centre [20].

Inhibition of AChE by aluminium was of a noncompetitive type, and the obtained inhibitor dissociation constant (K_i = 1.56 mM) is practically the same as that found by Sharp and Rosenberry [19] (1.5 and 1.7 mM), but is higher than that described by Marquis and Lerrick [8] (0.02 to 0.05 mM). As for defining the type of activation [21], that of low aluminium concentration is noncompetitive with the substrate.

Noncompetitive inhibition as well as activation is assumed to involve a reaction of the ligand with a region other than the active center with no change in the affinity of the catalytic site for the natural substrate acetylcholine. The activation of AChE by Al^{3+} is entirely allosteric, and Al^{3+} binds to the β-anionic site of the enzyme. The Hill coefficient (n = 1.34) indicates a slightly positive cooperative binding of aluminium. The β-anionic site is formed by the carboxyl group of glutamic acid [22] and is most likely localized in some polypeptide chain other than that of the α-anionic site which, together with the esteratic site, forms the so-called catalytic center [23]. The binding of ligands to the β-anionic site is based on electrostatic interactions and induces conformational changes that increase or decrease catalytic activity [16, 24]. The β-anionic site binds atropine [15], Ca^{2+} [16], 9-amino-10-methyl-1,2,3,4-tetrahydroacridinium [25], and also Al^{3+}, as we demonstrated in this paper. The transition from activation to inhibition of the AChE as a function of Al^{3+} concentration may be due to the heterogeneity of β-anionic sites, classified in classes of high-affinity and low-affinity sites [26]. Concentration-dependent effects of some lanthanides on the AChE activity were also observed [25, 27, 28].

The observation that aluminium may bind to the AChE in a physiological medium and the assumption that it may influence the enzyme activity ***in situ*** supports the suggestion that the high Al^{3+} levels observed in some brain regions of the patients with senile dementia of the Alzheimer type [3] may impair the cholinergic neurotransmission.

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