

Inhibition of Cholinesterase by Dialkylcarbamates

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The pI_{50} index and separation coefficients of chosen 3-*N,N*-diethylaminophenyl-*N,N'*-dialkylcarbamates were determined. Index pI_{50} (pI_{50} = negative logarithm of molar concentration of inhibitor inhibiting the enzyme activity by 50%) describes the effectiveness of the inhibitor. The rate of ability of the inhibitor to pass the blood-brain barrier is usually described by the separation coefficient in a system *n*-octanol/water (K_{ow}). Obtained results were compared with pI_{50} and K_{ow} of Exelon[®], the commercially used drug against the Alzheimer's disease.

Key words: Cholinesterase Inhibitors, pI_{50} , K_{ow}

Introduction

Our research group studies carbamate inhibitors of cholinesterases since several years. In our earlier works we were engaged in monoalkylcarbamates (Zdražilová *et al.*, 2004, 2006). This work is focused on dialkylcarbamates.

The aim of our research is to find a suitable inhibitor decreasing the activity of acetylcholinesterase (ACHE) and butyrylcholinesterase (BCHE) and to compare its inhibiting power (described by the index pI_{50}) and separation coefficient with the drug against Alzheimer's disease (AD) – Exelon[®].

Acetylcholine acts as a neuromediator of the cholinergic nerve system in the human brain. The decreased amount of acetylcholine is considered to be one of the most common reasons for AD (Scheibel *et al.*, 1986).

Acetylcholine is hydrolyzed by several forms of cholinesterases, which can be inhibited by various

inhibitors. Carbamates belong to the most important cholinesterase inhibitors. They are synthetic analogues of acetylcholine. The inhibition effect of carbamates is based on their ability to react with the reaction centre of acetylcholinesterase, which is formed by the hydroxy group of serine. These inhibitors block physiological functions of ACHE by carbamoylation of the serine hydroxy group (Booth and Metcalf, 1970).

Experimental

Chemicals

Dioxane p. a., KH_2PO_4 p. a., $Na_2HPO_4 \cdot 12 H_2O$ were all from Lachema, Brno, CZ.

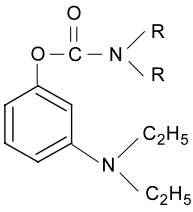
Acetylthiocholine iodide p. a. (substrate) was kept at 5 °C. From this substance the analytical aqueous solution (0.225 mM) was prepared for daily experiments and kept at 5 °C. Acetylcholinesterase: ACHE1, lyophilisate from electric eel; ACHE2, lyophilisate from bovine erythrocytes. Butyrylcholinesterase: BCHE, lyophilisate from equin serum. All enzymes were kept at –6 °C. A suitable amount of every enzyme preparation was dissolved in demineralized water (the enzymatic activity of these solutions was 0.25 U/ml). This solution was divided into suitable aliquots which were kept frozen at –6 °C. For the daily experiments a portion was melted and kept at 5 °C.

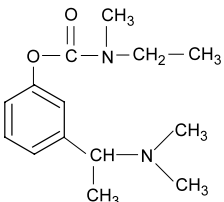
5,5'-Dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) was kept at 5 °C. From this substance the analytical aqueous solution (0.21 mM) was prepared and kept at 5 °C. Substrate, enzymes and Ellman's reagent were obtained from Sigma-Aldrich, Praha, CZ. The used inhibitors (Table I) were synthesized in the Department of Organic Chemistry, Faculty of Chemical Technology, University of Pardubice, Czech Republic. All inhibitors were kept at 5 °C. Inhibitors were first solved to 0.05 M solutions in dioxane and then diluted to smaller concentrations by demineralized water.

Method and apparatus

Determination of the index pI_{50}

Into the test tubes 0.1, 0.2, 0.3, 0.4 and 0.5 ml of 5 mM, 0.5 mM, 0.05 mM and 5 μ M solution of the tested inhibitors I–IV (Table I) were placed. All solutions were filled to the volume of 1 ml with phosphate buffer, pH 7.6. One test tube contained

	Carbamates	R	Nomenclature
I	CH ₃		3- <i>N,N</i> -Diethylaminophenyl- <i>N',N'</i> -dimethylcarbamate
II	C ₂ H ₅		3- <i>N,N</i> -Diethylaminophenyl- <i>N',N'</i> -diethylcarbamate
III	C ₃ H ₇		3- <i>N,N</i> -Diethylaminophenyl- <i>N',N'</i> -diisopropylcarbamate
IV	C ₄ H ₉		3- <i>N,N</i> -Diethylaminophenyl- <i>N',N'</i> -dibutylcarbamate

	Exelon		(<i>S</i>)- <i>N</i> -Ethyl- <i>N</i> -methyl-3-[1-(dimethylamino)ethyl]-phenylcarbamate hydrogen-(2 <i>R</i> ,3 <i>R</i>)-tartrate
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. C₄H₆O₆

Table I. An overview of used inhibitors.

only 1 ml of phosphate buffer, pH 7.6. Then 0.5 ml of enzyme preparation (ACHE1, ACHE2 or BCHE) was added into all test tubes. Mixtures were quickly homogenized and then thermostated 5 min at 25 °C. Then 0.25 ml of acetylthiocholine (0.225 mM) and 0.25 ml of DTNB (0.21 mM) were added into all test tubes. Mixtures were quickly homogenized again and thermostated 10 min at 25 °C. Absorbance *A* (at 412 nm) of all mixtures was measured and compared with a comparative solution (1 ml of phosphate buffer, pH 7.6, 0.5 ml of demineralized water, 0.25 ml of 0.225 mM acetylthiocholine and 0.25 ml of 0.21 mM DTNB). From the dependence inhibition of enzyme preparation in percent (%) [*I*] [calculated according to (1)] vs. *pI* (= -log [*I*]) the value of the index *pI*₅₀ was determined graphically:

$$\%I = \left(1 - \frac{A_i}{A_0}\right) * 100, \quad (1)$$

where *A*_i is the absorbance of solution with inhibitor and *A*₀ is the absorbance of solution without inhibitor.

Determination of the separation coefficient (*K*_{ow}) in the system *n*-octanol/water

Into the test tube 1.5 ml of *n*-octanol were placed. Then 10 μl of octanol solution of the chosen inhibitor (0.05 M) were added. Mixture was intensively shaken for 15 min. 1 ml of this mixture was placed into the cuvette and its absorbance at the wave-

length of absorption maximum was measured. Comparative solution was *n*-octanol. The value of absorbance which corresponds to 100% of chosen inhibitor in *n*-octanol was obtained.

10 μl of octanol solution of chosen inhibitor (0.05 M) were added into the mixture of *n*-octanol (1.5 ml) and demineralized water (1.5 ml). Mixture was intensively shaken for 15 min and then centrifuged (3000 rpm, 10 min). 1 ml of the octanol layer was put into the cuvette and its absorbance at the wavelength of absorption maximum was measured. Comparative solution was *n*-octanol again. Percentage content of the chosen inhibitor in the octanol layer (%)₁ was obtained. Then the percentage content of inhibitor in the water layer was calculated [100 - (%)₁]. Separation coefficients were calculated according to

$$K_{ow} = \frac{(\%)_1}{100 - (\%)_1} = \frac{c_1}{c_2}, \quad (2)$$

where *c*₁ and *c*₂ are molar concentrations of the chosen inhibitor in *n*-octanol and water.

Results and Discussion

In Table II the values of the index *pI*₅₀ and separation coefficients *K*_{ow} of the tested inhibitors are presented.

From the obtained results we can formulate the following conclusions:

Inhibitor	pI_{50} (ACHE1)	pI_{50} (ACHE2)	pI_{50} (BCHE)	K_{ow}
I	4.61	4.22	5.74	3.12
II	4.05	3.13	6.44	1.87
III	4.13	3.35	4.84	0.86
IV	4.76	3.65	4.92	0.98
Exelon®	3.26	4.15	4.75	0.09

Table II. The determined values of index pI_{50} and separation coefficients of tested inhibitors.

1. All tested substances are at least the same effective inhibitors as the used drug against Alzheimer's disease – Exelon®.
2. Substitution by a longer alkyl group on the N atom hasn't a uniform influence on the value of the index pI_{50} .
3. The most effective inhibitor of ACHE1 is inhibitor IV, of ACHE2 inhibitor I and of BCHE inhibitor II.
4. Generally it is possible to conclude that inhibition of BCHE by the used inhibitors is stronger than of ACHE1 and ACHE2.
5. In comparison with Exelon® (Zdražilová *et al.*, 2004, see Table II) the presented index pI_{50} values of the tested substances exhibit the same or even better inhibition of the used enzymes *in vitro*.
6. The values of separation coefficients show a uniform effect. The value of separation coefficient decreases with increasing number of C atoms of the alkyl group. The exception is only inhibitor III, because its K_{ow} value is lower than the K_{ow} value of inhibitor IV.

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