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Copper, aluminum, iron and calcium inhibit human acetylcholinesterase in vitro

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ABSTRACT

Acetylcholinesterase (AChE) is an important part of cholinergic nerves where it participates in termination of neurotransmission. AChE can be inhibited by e.g. some Alzheimer disease drugs, nerve agents, and secondary metabolites. In this work, metal salts aluminum chloride, calcium chloride, cupric chloride, ferric chloride, potassium chloride, magnesium chloride and sodium chloride were tested for their ability to inhibit AChE. Standard Ellman assay based on human recombinant AChE was done and inhibition was measured using Dixon plot. No inhibition was proved for sodium, potassium and magnesium ions. However, aluminum, cupric, ferric and calcium ions were able to inhibit AChE via noncompetitive mechanism of inhibition. Though the inhibition is much weaker when compared to e.g. drugs with noncompetitive mechanism of action, biological relevance of the findings can be anticipated.

1. Introduction

Currently, two enzymes hydrolyzing esters of choline are known. Acetylcholinesterase (AChE; EC 3.1.1.7.) is an enzyme hydrolyzing neurotransmitter acetylcholine and it can be mentioned as the first. Butyrylcholinesterase (BChE; EC 3.1.1.8.) is the second enzyme able to hydrolyze choline esters (Pohanka, 2011, 2012b,c). AChE plays an important role in cholinergic neurotransmission where it terminates stimulation in neurosynaptic cleft by hydrolyzing of acetylcholine (Wessler and Kirkpatrick, 2008). BChE is abundantly presented in plasma and tissues; however, its biological role is not well understood (Masson et al., 2009). The most significant effect of BChE in the body is detoxification of some drugs including procaine and muscle relaxant succinylcholine (Yuan et al., 2007).

The both cholinesterases have large structural similarities. They are a type α/β hydrolase folded and the both contains Ser-His-Glu in its active site (Cygler et al., 1993). The active site is localized in a cavity with peripheral (or β) anionic site in its entrance followed by aromatic gorge. As the last part, α anionic site responsible for proper orientation of substrate toward the catalytic triade can be mentioned (Macdonald et al., 2012).

Cholinesterases can be inhibited by many compounds including drugs, natural toxins, military used nerve agents and others. Inhibitors binding into active site of cholinesterases have typically equal affinity toward AChE and

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BChE. Nerve agents sarin, VX and some pesticides such as carbofuran can be exampled (Knaack et al., 2012; Pohanka, 2011). Different situation can be learned for inhibitors binding into aromatic gorge or peripheral anionic site which are more extensively developed in AChE than in BChE (Macdonald et al., 2012).

Cholinergic system and activity of AChE can be influenced by heavy metals as proved using some in vivo models (Gioda et al., 2013; Saidi and Shojaie, 2012). The effect is not; however, well understood. In this work, an attempt to identify one of the possible molecular mechanisms where heavy metals are involved in cholinergic system was made. In order to identify molecular mechanism, affinities of three biologically and toxicologically relevant metals: calcium, copper, iron, sodium, magnesium and aluminum to AChE were measured. The chosen metals are quite common and they are occurring as either biogenic elements or elements with high occurrence in environment. Salts of all these elements are soluble in water so the assay is well reproducible and is not sensitive to a methodical error. Beside this, the good solubility in water is an important attribute that the compound will be easily distributed in the body which is necessary for a biological impact related to cholinesterases. Possibility that the metals act as inhibitors is hypothesized prior to the experiment.

2. Material and methods

2.1. Reagents

In the experiment, human recombinant AChE (expressed in HEK 293 cells, lyophilized powder ≥1500 U/mg of protein) was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). 5,5′-Dithiobis-(2-nitrobenzoic) acid and acetylthiocholine chloride were received from Sigma-Aldrich as a chromogen and substrate in purity at least 98%. The enzymes, thiocholine esters and 5,5′-dithiobis-(2-nitrobenzoic) acid were dissolved in phosphate buffered saline pH 7.4 with composition 137 mmol/l NaCl, 2.7 mmol/l KCl, 10.0 mmol/l Na_2HPO_4, 0.240 mmol/l KH_2PO_4 (Penta-Chemicals, Prague, Czech Republic). Aluminum chloride ($AlCl_3$), calcium chloride ($CaCl_2$), cupric chloride ($CuCl_2$), ferric chloride ($FeCl_3$), potassium chloride (KCl), magnesium chloride ($MgCl_2$) and sodium chloride (NaCl) were received in high purity (above 99%) from Sigma-Aldrich as well. The salts were solved in deionized water with conductivity 6 μS/m prepared by reverse osmosis by Aqua Osmotic 02 instrument (Aqua Osmotic, Tisnov, Czech Republic).

2.2. AChE activity assay

Standard spectrophotometric test for AChE activity assay was performed in compliance with cited papers (Pohanka, 2012a, 2013). In the assay, acetylthiocholine was used as a substrate for AChE. Standard polystyrene disposable cuvettes with optical length 1 cm were used for purpose of the assay. One cuvette was filled with 0.4 ml of 5,5′-dithiobis-(2-nitrobenzoic) acid 1 mmol/l, 100 μl of AChE solution (activity in the 100 μl solution adjusted up 2×10^{-10} kat for 1 mmol/l substrate and SATP conditions), 100 μl of the tested compound solution and 300 μl of phosphate buffered saline. The tested compound was adjusted in two times diluted calibration scale ranging from 50 to 500 mmol/l. The reaction was started by addition of acetylthiocholine chloride (100 μl). Five minutes after the substrate injection to cuvette, absorbance was measured at 412 nm. Enzyme activity was calculated using extinction coefficient $\varepsilon = 14{,}150\,l \times mol^{-1} \times cm^{-1}$ that is valid for the used assay conditions (Eyer et al., 2003).

2.3. Statistical processing of experimental data

All measurements were repeated five times and Dixon plot was used for data processing in a way as described by Cornish-Bowden (1973). The data were plotted by two ways. First, reciprocal value of velocity was plotted against inhibitor concentration. Second, substrate concentration divided by reaction velocity was plotted against inhibitor concentration. The inhibition constants K_i were calculated from the plots by a standard manner (Cornish-Bowden, 1973; Dixon, 1953). Significance of difference between two sets of measurements was calculated using analysis of variance (ANOVA) tests on the both P 0.05 and P 0.01 levels.

3. Results and discussion

Sodium chloride, magnesium chloride and potassium chloride caused no inhibition of AChE. The activity measured for control assay was not differing (tested by ANOVA on the both probability levels) to activity of AChE in presence of the upper concentration of sodium, potassium or magnesium. The finding is not surprising. In the body, the metals are dissolved and presented in bulky concentration in free, solved ionic form (Aramli et al., 2013; Asadi et al., 2010; McCallum et al., 2013). For the reason, the ions cannot act as inhibitors because it would lead to unwanted suppression of the enzymes activity.

Contrary to the aforementioned metals, aluminum chloride, calcium chloride, cupric chloride and ferric chloride were potent enough to significantly inhibit AChE. Dixon plots are depicted as Fig. 1 for aluminum chloride, Fig. 2 for calcium chloride, Fig. 3 for cupric chloride, and Fig. 4 for ferric chloride. Considering mechanism of action, noncompetitive inhibition was proved for the four mentioned metals. Chloride presented in the salts probably plays no role in the inhibition mechanism. The salts are fully dissociated in the used pH. If chloride has a role, it had to be proved in the assay of sodium chloride, magnesium chloride and potassium chloride but the contrary is true. From the tested salts, the highest inhibition was caused by cupric ion as inhibition constant K_i was equal to 781 ± 69 μmol/l. The last three inhibiting metal ions had inhibition constant lower. It was 5.46 ± 0.58 mmol/l for aluminum ion, 14.5 ± 1.13 mmol/l for calcium ion, and 22.1 ± 1.3 mmol/l for ferric ion. The found K_i values are summarized in Table 1 together with chosen physical and physico-chemical parameters tabulated for the metals. As seen, the difference in ability to inhibit AChE by the tested metals is quite huge. Copper has 28.3 times higher affinity toward AChE than the weakest inhibitor, ferric ion. On the other hand, the metals are significantly weaker inhibitors when compared to some standard drugs and even the strongest inhibitor reported here, copper,