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PREFACE

Dear readers,

following is a set of lectures delivered at different Congresses, Symposia and Workshops. Some of these were invited, some of these were published in different forms. The main topics is clear from the content - Cholinergic nerve transmission and its influencing. The authors tried to see cholinergic nerve transmission from different angles, nevertheless, the focus is given to influence this transmission with nerve agents and other factors. I hope that this special issue of our Journal „Vojenské zdravotnické listy" will be useful and interesting for all workers in this field.

Editor

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CHOLINESTERASES AND THEIR POSSIBLE INFLUENCING

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Summary

Acetylcholinesterase and butyrylcholinesterase is characterized according to their properties and possible influencing of their activities by inhibitors and other factors.

KEY WORDS: Acetylcholinesterase; Butyrylcholinesterase; Characterization; Inhibitors; Other factors.

Cholinesterases belong to the group of hydrolases catalyzing hydrolysis of choline esters to choline and relevant acid. They are distinguished into two classes, differing in their enzymatic properties and physiological function: acetylcholinesterase (AChE, EC 3.1.1.7.) and butyrylcholinesterase (BuChE, EC 3.1.1.8.) as it is shown in Table 1. However, there exist other types of cholinesterases like benzoylcholinesterase, propionylcholinesterase etc.

Activity of cholinesterases is of fundamental importance for diagnosis of intoxication with cholinesterase inhibitors, including organophosphates (OP) and carbamates (5, 17, 26). On the other hand, the activity depends on many other factors and therefore cholinesterase determination is of diagnostic importance in different pathological states, i.e. not only intoxications (7, 8, 11, 13, 14, 35). Activity of both enzymes (AChE and BuChE) is influenced by sex, age, nutrition, hormonal factors,

Table I

Differences between properties of AChE and BuChE

Trivial name	Acetylcholinesterase	Butyrylcholinesterase
Other names	specific, true, „e" type cholinesterase	cholinesterase, pseudocholinesterase, „s" type cholinesterase
Systemic name	acetylcholine-acetylhydrolase	acetylcholine-acylhydrolase
Number of Enzyme		
Codex	EC 3.1.1.7.	EC 3.1.1.8.
Source	electric, organ, brain, erythrocytes	serum, plasma
Optimal substrate	acetylcholine	butyrylcholine
Splitting acetyl-β-methylcholine	yes	no
Species differences	low	high
Inhibition by Substrate	yes	no
Quaternary ammonium salts	+ + +	+
Iso-OMPA	+	+ + +
Phenothiazines	+ + +	+
Tacrine	+	+ + +
DMC	+ + +	+
Ni, Zn	+ +	+
Binding	complex with lipoprotein	glycoprotein; containing sialic acid
Molecular forms	subunits	genetically determined
Activation Mg, Mn	Mg > Mn	Mg < Mn
Function	splitting neuromediator acetylcholine	unknown

irradiation etc. (15, 23, 37, 38, 45). The variation of BuChE activity is greater than that of AChE (5, 7).

In the clinical biochemistry, BuChE determination in the plasma or serum is more frequently used than that of AChE in the red blood cells. Except intoxication with OP or carbamates, BuChE decrease indicates either diminution of synthesis of the enzyme or the decrease of the number of production cells in the liver (27). Special case of diminished BuChE activity is hereditary affected presence of atypical variants of BuChE (16, 45).

It was demonstrated by Whittaker (45) a qualitative difference between BuChE of suxamethonium sensitive individuals and that of other patients. The two types of enzyme hydrolyse the same substrate at different rates and show distinct inhibition with varying concentrations of suitable inhibitor such as dibucain. These findings were the basis of the hypothesis that the biosynthesis of BuChE is controlled by two allelic genes, E^{u}_{1} and E^{a}_{1}. Individuals with combination $E^{u}_{1}E^{u}_{1}$ are homozygotes with normal BuChE activity; combination of $E^{a}_{1}E^{u}_{1}$ (heterozygotes) and $E^{a}_{1}E^{a}_{1}$ (homozygotes) resulted in diminished BuChE activity. The presence of a silent gene (E^{s}_{1}) was also proposed and the fourth gene controlling biosynthesis of BuChE (fluoride resistant, E^{i}_{1}) was recognised; the hypothesis was in general established by family studies (5, 15, 45).

The influencing of BuChE activity in gamma-irradiation, stress, gravidity, some neurologic and psychiatric disorders, hormones and medical drugs was demonstrated (5, 15). Elevation of BuChE activity is not so frequent; the increase in children with nephritic syndrome was observed (45); elevated ratio BuChE/LDL cholesterol indicates an increase of the risk of cardiovascular diseases (24, 29).

Determination of AChE activity is not so widely used in clinical laboratories. The decrease of the red blood cells AChE activity in pernicious anaemia was demonstrated; diminished erythrocyte AChE activity is typical for paroxysmal nocturnal haemoglobinaemia and ABO incompatibility (35).

Activity of AChE in the erythrocyte membrane can be considered as an indicator of integrity of erythrocyte membrane. Increased AChE activity in rectal biopsy is of great diagnostic significance in Hirschsprung's disease, especially the presence of its atypical molecular form (8, 35). There are other papers demonstrating increased AChE activity in the amniotic fluid during pathologic development of neural tube (13). AChE activity in the erythrocytes and cerebrospinal fluid is diminished also in some endogenous depressions and other psychiatric disorders; however, the results presented are not quite clear at present (for review see, e.g. 5, 35, 37).

On the other hand, influencing of cholinergic nervous system is one of the most important pathological changes in Alzheimer's disease (2). A lack of cholinergic mediator, acetylcholine, was observed (32). This had led to attempts to correct cholinergic deficiency at various levels of choliner-

gic functioning: inhibitors of cholinesterases like physostigmine were used; however, physostigmine was not found to be an ideal drug for clinical use because of its short half-life, side-effects etc. (19). The results of clinical studies showed perspective results with acridine inhibitor of cholinesterase - Tacrin (1,2,3,4-trahydro-9-aminoacridine) (40); its 7-methoxyderivative (7-MEOTA) was described as a compound of low toxicity and good therapeutic effect in experimental intoxication with anticholinergics (20). Biochemical studies dealing with its effect on cholinesterases in vivo showed that 7-MEOTA inhibited BuChE in the liver and AChE in the brain parts with the highest sensitivity for frontal cortex (7).

There are many inhibitors of cholinesterases diminishing both AChE and BuChE activities to a comparable extent. However, there is a number of important exceptions: the selectivity of some OP and carbamates for BuChE has been described by Aldridge (1) and reviewed by other authors (5, 15, 45). Carbamates belong to the group of insecticides with a large variety of their effectiveness. They are biologically active because of their structural complementarity to the active surface of AChE and their consequent reaction as substrates with very low turnover numbers (1, 5, 19). Some carbamates inhibit selectively either AChE or BuChE (7, 9). Toxicity of carbamates is dependent on their ability to carbamylate AChE in different tissues and on other factors, i.e. distribution, detoxification, metabolization etc. 3-Diethylaminophenyl-N-methylcarbamate methiodide belongs to highly toxic carbamates (7). The selectivity for the both cholinesterases in vivo was not so expressed as it was demonstrated in experiments in vitro (7).

The interesting group of compounds modifying cholinesterase activity are metal cations. Their effect was studied on relatively simple cholinesterase models and these studies were finished in latest years. In connection with further work dealing with the active surface of cholinesterases, especially AChE, these works were found in literature because metal's ability to interact with different active sites on AChE. These studies are very limited and hardly comparable because of their different methodical approach. It was demonstrated previously that especially Hg ions diminished AChE activity in low concentrations (7).

The factors influencing cholinesterases are not limited to chemicals only. AChE activity is connected with cholinergic activity in the brain. Using determination of AChE combined with defined lesions of different parts of the brain, it was possible to demonstrate cholinergic projections in the central nervous system.

The septum is known to be an important part of the limbic system. Functional relationship between the septum and other limbic structures have been demonstrated in several behavioural, electrophysiological, biochemical and histochemical studies. These regions contain neurones which appear to be selectively sensitive to cholinergic and anticholinergic drugs (23). It has been demonstrated previously that septal nuclei are reciprocally connected with different brain areas - especially with the hippocampus (23). The changes of AChE activity in various parts of the limbic system produced by lesions of the medial septum suggest connections of medial septum with hippocampus, hypothalamus and ncl. amygdalae. Dorsal septum has cholinergic projections with hippocampus, thalamus, and frontal cortex (7, 23).

Both enzymes (AChE and BuChE) exist in multiple molecular forms (14, 35). The activities of these forms are also influenced by many factors. The function of these forms is not known at present; however, their presence in the membrane structures at physiological conditions was demonstrated (6). There are only scarce data describing the changes of AChE molecular forms following intoxication with highly toxic OP (3, 25). Some experiments were performed with relatively less toxic OP (7, 9, 10, 16, 28, 30). From the group of highly toxic OP compounds, sarin, soman, and VX were found to be the most effective (3).

Molecular forms of AChE showed different sensitivity to inhibitors in vitro (9, 30) and in vivo (3, 10, 16, 25). Following DFP (28) and highly toxic OP (3, 25), the form with high molecular weight was the most sensitive. Intoxication with Parathion and Neguvon (less toxic OPs) caused medium inhibition of some forms of AChE (7, 16).

From different results describing multiple molecular forms of AChE it can be concluded that AChE in the brain exists in molecular forms. These forms were observed also by other authors (3, 6, 7, 28). These forms are different for various species. However, the electrophoretic mobility of AChE components from the rat, rabbit, mouse and human brains suggested that there are generally two types of AChE forms having high and low molecular weight. One BuChE and two AChE bands in the rat hippocampus after electrophoresis in polyacrylamide gel were observed (42). The distinction between the two AChE forms is difficult without electrophoresis. They differ in electrophoretic mobility and they can be well differentiated by electrophoresis.

Subcellular localization of AChE suggested that in nerve ending particles and microsomal fractions 2-4 AChE forms are present, in the mitochondrial fraction only one was detected (4). The microsomal form absent in the mitochondrial fraction is the most sensitive to OP in vivo. From previous studies it is known that high molecular form of AChE has the lowest K_m value (12) and highest decrease in this component after deafferentation was also observed (11). These results suggested that this form of AChE would be very important for normal

cholinergic nerve transmission. It arises the question on existence of the forms under physiological conditions. Using thermal denaturation, it was demonstrated that they are not artifacts formed during homogenization or other treatment of the brain tissue (6). The overall data show that catalytic activity of AChE molecular forms is different and their inhibition by various inhibitors may be heterogeneous. This heterogenicity was demonstrated for AChE phosphorylating inhibitors as well as for inhibitors with different binding sites for the enzyme.

The results with another type of inhibitor - 7-MEOTA - fit well with our previous findings indicating a greater sensitivity of slowly migrating molecular forms separated by polyacrylamide gel electrophoresis (7). In fact, it has been demonstrated recently that slowly migrating forms of cortical AChE correspond to G_4 forms separated by sedimentation analysis (43). On the other hand, recent data indicate an almost equal sensitivity of G_4 and G_1 forms of both soluble and membrane-bound whole brain AChE to this type of inhibitor (30). It is not excluded that the reversible inhibitors such as 7-MEOTA modify their interaction with the active site resulting in a preferential inhibition of G_4 forms. It is of interest that the introduction of a heptyl group into physostigmine modified its interaction with the AChE molecular forms, heptyl-physostigmine showing a stronger inhibition for G_1 than for G_4 forms while in the case of the parent compound similar inhibition of the two forms was observed (30).

The data of 7-MEOTA are different from those obtained for DFP and paraoxon showing similar IC_{50} values for G_4 and G_1 forms (43). These findings have been confirmed for membrane-bound AChE (30). This is not surprising since the interaction of OP compounds (and physostigmine) with the active site of enzymatic molecule is different from that for 7-MEOTA-type compounds. In fact, OP compounds inhibit AChE by phosphorylating the esteratic serine in the catalytic site. On the other hand, acridine derivatives bind to the hydrophobic area close to the active site of AChE simultaneously affecting its catalytic center via an allosteric mechanism (18, 31, 39).

As regards the data on AChE molecular forms, they confirm previous findings indicating a more pronounced sensitivity of G_4 forms, as compared to G_1 forms, in brain of rats injected with paraoxon (43). Somewhat lower inhibitory effects of the same dose of paraoxon (0.25 mg/kg s.c.) as well as a somewhat lower contribution of G_4 forms to total AChE in untreated rats were observed in another experiments (10) in comparison with those reported by Volpe et al. (43) and may depend on regional differences (cerebral cortex and whole brain).

In the case of brain AChE, as it has been pointed out (43), G_4 and G_1 forms represent distinct pools in the cell, the former being mainly associated with membranes, with its catalytic site exposed to the extracellular space, and the latter confined to the intracellular compartment.

Following intoxication with nerve agents mentioned, the highest sensitivity for high molecular AChE form was observed (3). Determination of the whole AChE activity is partly misrepresenting because of different distribution of AChE molecular forms in the sample. Following determination of the whole activity, a „mean" activity containing activities of the forms is determined. It can be concluded that in studies requiring high sensitivity (e.g. the studies of antidotal action), AChE molecular forms would be of choice for more detailed information of functional stage of AChE - important marker of cholinergic nerve transmission.

The three-dimensional structure of AChE from Torpedo californica electric organ was determined in 1991 by Silman et al. (41). The enzyme monomer is an $\alpha\beta$ protein that contains 537 amino acids. It consists of a 12-stranded mixed β-shift surrounder by 14 α-helixes and bears a striking resemblance to sever hydrolase structures. Like other serine hydrolases, it contains a catalytic triad at the bottom of a deep and narre cavity, known as the „aromatic gorge" since more than 50 of its lining is composed of the rings of 14 conserved aromatic amino acids. ACh must pass down into this gorge bind to the active site within. Manual docking of the substrate AChE at the base of the gorge reveails the esteratic and choline binding sites.

The esteratic site is the catalytic triad formed by Ser_{200}-His_{440}-Glu_{327}, in which the Glu_{327} stabilizes the His_{440} tautomer required for general acidbase function and/or electrostatically stabilizes the incipient His_{440} imidazolium cation during catalysis (33). Associated with the triad, a putative oxyanion hole formed by the two amidic nitrogens of Gly_{118} and Gly_{119} has been identified. Modelling of the quaternary group of ACh at the active site suggests that it forms a cation-πbond with the indole of conserved Trp_{84}, in agreement with affinity labelling experiments (44).

Very recently the native structure of T. californica AChE, determined to a resolution of 2.5Á, was reported (36). The docking study with ACh modelled in this structure showed that the acetate moiety retains its position near Ser_{200} and that the quaternary nitrogen is positioned at 4.8Á from the centroid of the entire 9-membered ring of Trp_{84}, so that the distance from the phenyl ring of Phe_{330} is 5.2Á. Thus, the quaternary ammonium group expresses two cation-aromatic interactions.

It is interesting to compare the structures of AChE and BuChE. These two enzymes possess 53% sequence homology (21), which has permitted the modelling of BuChE on the basis of the 3D

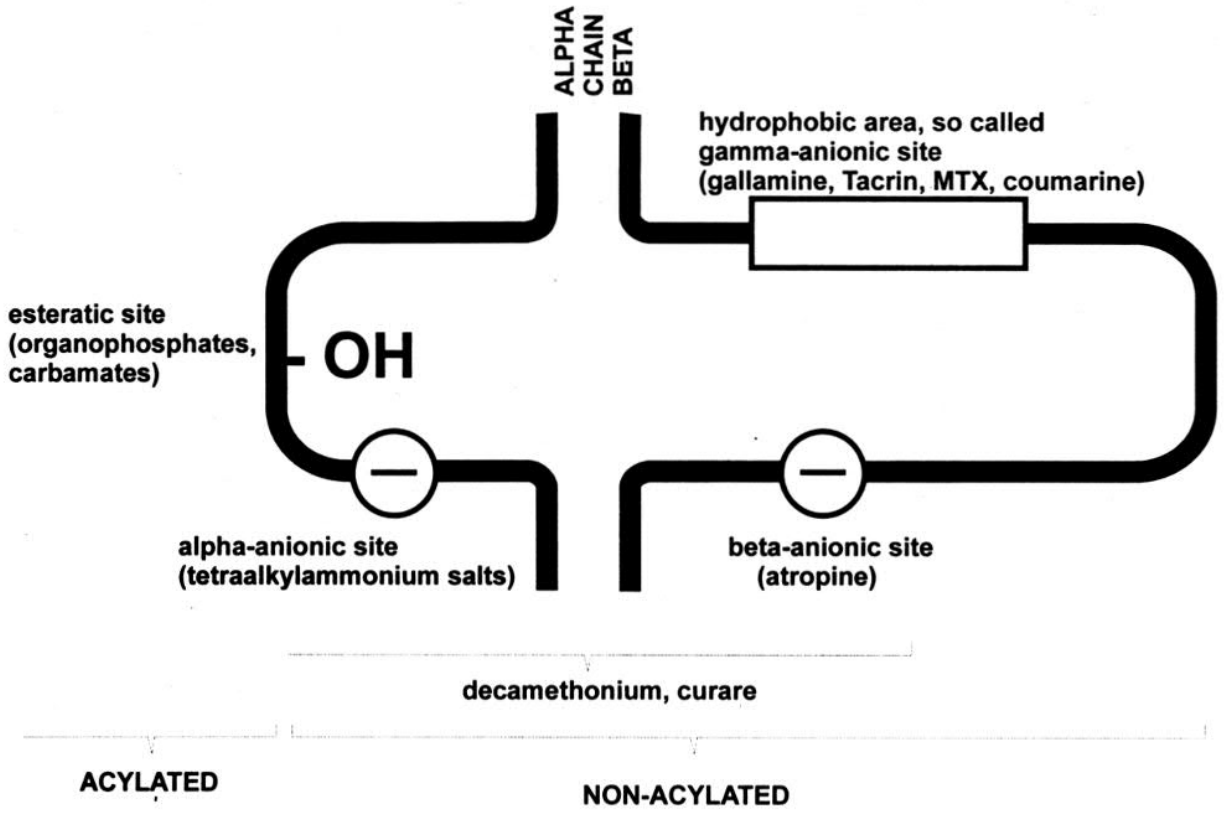

Fig. 1 Hypothetic structure of active surface of AChE including different binding sites and differentiation to acylating and non-acylating inhibitors

structure of Torpedo AChE (22). Six aromatic residues which are conserved in AChE and line the gorge leading to the active site are absent in BuChE. Computer modelling has shown that two such residues, Phe_{288} and Phe_{290}, which are replaced in BuChE by leucine and valine, respectively, may prevent bulky esters of choline from entering into the acyl-binding pocket of AChE. Mutagenicity experiments have confirmed this model.

In addition to the subsites of the catalytic center, AChE possesses one or more additional binding sites for ACh and other quaternary ligands. Such peripheral anionic binding sites are at the lip of this gorge. In BuChE, Trp_{279}, an important component of the peripheral binding site in AChE, is missing. This site is believed to be responsible for substrate inhibition (34), which is one of the features that distinguishes AChE from BuChE. Recently it was suggested that the peripheral anionic site affected by Alzheimer's disease loses their substrate inhibition specificity (2).

Very schematically, in Fig. 1, is shown a model of hypothetic structure of AChE including binding sites and differentiation of acylating and non-acylating inhibitors.

These studies are necessary for elucidation of action of inhibitors on AChE. This approach could only improve our knowledge of mechanisms of action of OP and other inhibitors and of the poisoning caused by these chemicals and their treament. Simultaneously, it could contribute to better understanding of cholinergic nerve transmission and thus to pharmacology and neuropharmacology in general.

References

1. ALDRIDGE, WN.: Organophosphorus compounds and carbamates, and their reactions with esterases. Brit. med. Bull., 1969, vol. 25, p. 236-239.
2. ARENDT, T., et al.: Changes in acetycholinesterase and butyrylcholinesterase in Alzheimer's disease resemble embryonic development - A study of molecular forms. Neruchem. Int., 1992, vol. 21, p. 381-396.
3. BAJGAR, J.: Differential inhibition of the brain acetylcholinesterase molecular forms following soman, sarin and VX intoxication in laboratory rats. Acta Medica (Hradec Králové), 1997, vol. 40, p. 89-94.
4. BAJGAR, J.: Characterization and properties of multiple molecular forms of acetylcholinesterase. Possible evaluation in histochemistry. Acta Histochem., 1975, Suppl. 15, p. 123-132.
5. BAJGAR, J.: Intoxikace organofosforovými inhibitory cholinesteráz: účinek, diagnóza a terapie. Novinky v medicíně. Praha, Avicenum, 1985, roč. 34, s. 7-40.

6. BAJGAR, J.: Isoenzymes of rat brain acetylcholinesterase: facts or artifacts? J. Neurochem., 1979, vol. 32, p. 1875-1879.
7. BAJGAR, J.: The influence of inhibitors and other factors on cholinesterases. Sbor. ved. Prací lék. Fak. UK Hradec Králové, 1991, vol. 34, p. 3-75.
8. BAJGAR, J. - HAK, J.: Acetylcholinesterase activity and its molecular forms in rectal tissue in the diagnosis of Hirschprung's disease. Clin. Chim. Acta, 1979, vol. 93, p. 93-95.
9. BAJGAR, J. - MICHALEK, H. - BISSO, GM.: Differential inhibition of rat brain acetylcholinesterase molecular forms by 7-methoxytacrine in vitro. Toxicol. Lett., 1995, vol. 80, p. 109-114.
10. BAJGAR, J. - MICHALEK, H. - BISSO, GM.: Differential reactivation by HI-6 in vivo of Paraoxon-inhibited rat brain acetylcholinesterase molecular forms. Neurochem. Int., 1995, vol. 26, p. 347-350.
11. BAJGAR, J. - PAŘÍZEK, J.: Changes of acetylcholinesterase and esterase isoenzymes in isolated cerebellar cortex of the rabbit. Brain. Res., 1973, vol. 57, p. 299-305.
12. BAJGAR, J. - ŽIŽKOVSKÝ, V.: Partial characterization of soluble acetylcholinesterase isoenzymes of the rat brain. J. Neurochem., 1971, vol. 18, p. 1609-1614.
13. BONHAM, JR. - ATTACK, JR.: A neural tube defect specific form of acetylcholinesterase in amniotic fluid. Clin. Chim. Acta, 1983, vol. 135, p. 233-237.
14. BRIMIJOIN, S. - RAKONCZAY, Z.: Immunocytology and molecular histology of cholinesterases: current results and prospects. Int. Rev. Neurobiol., 1986, vol. 28, p. 353-410.
15. BROWN, SS., et al.: The plasma cholinesterases: new perspectives. Adv. Clin. Chem., 1981, vol. 22, p. 1-123.
16. BROWNSON, RH., et al.: Parathion-induced alterations in chemical, biochemical and isoenzyme study. In L. Roizin (Eds.), Neurotoxicology, New York, Raven Press, 1977, p. 457-468.
17. ELLIN, RI.: Anomalies in theories and therapy of intoxication by potent organophosphorus anticholinesterase compounds. Gen. Pharmacol., 1982, vol. 13, p. 457-456.
18. FREEMAN, SE. - DAWSON, RM.: Tacrine: a pharmacological review. Prog. Neurobiol., 1991, vol. 36, p. 257-277.
19. FUSEK, J.: Tacrin a jeho analoga, antidota psychoatkivních látek s anticholinergním účinkem. Voj. zdrav. Listy, 1977, roč. 46, č. 1, s. 21-27.
20. FUSEK, J. - HRDINA, V.: Zneschopňující otravné látky s psychomimetickým účinkem. 1. vyd. Hradec Králové, VLVDÚ, 1984. Učební texty VLVDÚ JEP Hradec Králové. Sv. 206.
21. GENTRY, MK. - DOCTOR, PB.: Alignment of amino acid sequences of acetylcholinesterases and butyrylcholinesterases. In J. Massculié, et al. (Eds.), Cholinesterases: Structure, Function, Mechanism, Genetics and Cell Biologyet, American Chemical Society, 1991, p. 394-398.
22. HAREL, M., et al.: Conversion of acetylcholinesterase to butyrylcholinesterase: Modeling and mutagenesis. Proc. Natl. Acad. Sci. USA, 1992, vol. 89, p. 10827-10831.
23. HERINK, J. - BAJGAR, J. - PATOČKA, J.: Changes of acetylcholinesterase activity in some parts of the limbic system following septal lesions in rats. J. Neurochem., 1975, vol. 25, p. 187-188.
24. KUTTY, KM.: Biological function of cholinesterase. Clin. Biochem., 1980, vol. 13, p. 239-243.
25. Lenz, DE. - MAXWELL, DM.: Inhibition of rat cerebrum acetylcholinesterase isoenzymes after acute administration of soman. Biochem. Pharmacol., 1981, vol. 30, p. 1369-1371.
26. MARRS, TC. - MAYNARD, RL. - SIDELL, FR.: Chemical warfare agents. Toxicology and treatment. Chicester, New York, Brisbane, Toronto, Singapore, J. Wiley and Sons, 1996. 243 p.
27. MASOPUST, J.: Klinická biochemie jater žlučových cest. 2. část (Testy na tvorbu a vylučování žluči, testy na proteosyntézu a detoxikační funkci). Biochem. Clin. Bohemoslov., 1983, roč. 12, s. 363-376.
28. MICHALEK, H. - MENEGUZ, A. - BISSO, GM.: Molecular forms of rat brain acetylcholinesterase in DFP intoxication and subsequent recovery. Neurobehav. Toxicol. Terratol., 1981, vol. 3, p. 303-312.
29. NAVRÁTIL, L. - BAJGAR, J.: Vzájemná závislost aktivity BuChE v krevní plazmě a celkové cholesterolémie. Biochem. Clin. Bohemoslov., 1987, roč. 16, s. 117-121.
30. OGANE, N. - GIACOBINI, E. - MESSAMORE, E.: Preferential inhibition of acetylcholinesterase molecular forms in rat brain. J. Neurochem., 1992, vol. 17, p. 489-495.
31. PATOČKA, J. - BAJGAR, J. - BIELAVSKÝ, J. - FUSEK, J.: Kinetics of inhibition of cholinesterase by 1,2,3,4-tetrahydro-9--aminoacridine. Collect. Czech. Chem. Commun., 1976, vol. 41, p. 816-824.
32. PERRY, RH., et al.: Plasma and erythrocyte acetylcholinesterase in senile dementia of Alzheimer type. Lancet, 1982, vol. 1, p. 174-175.
33. PIGAR, L. - HULASZ, P.: Current problems in mechanistic studies of serine and cystine proteinases. Biochem. J., 1982, vol. 207, p. 1-10.
34. RADIC, Z. - REINER, E. - TAYLOR, P.: Role of the peripheral anionic site on acetylcholinesterase: Inhibition by substrates and coumarin derivatives. Mol. Pharmacol., 1991, vol. 39, p. 98-104.
35. RAKONCZAY, Z.: Cholinesterase and its molecular forms in pathological states. Progr. Neurobiol., 1988, vol. 31, p. 311-330.
36. RAVES, ML., et al.: Structure of AChE complexed with the nootropic alkaloid, (-) huperzine A. Nat. Struct. Biol., 1997, vol. 4, p. 57-63.
37. SKAU, KA.: Mammalian acetylcholinesterase:molecular forms. Comp. Biochem. Physiol., 1986, vol. 83C, p. 225-227.
38. SKOPEC, F. - BAJGAR, J. - PATOČKA, J.: Changes of acetylcholinesterase activity in different brain areas of the rat following head irradiation. Radiobiol. Radiotherap., 1981, vol. 22, p. 631-634.
39. SOREQ, H., et al.: Excavations into the active site-gorge of cholinesterase. TIBS, 1992, vol. 17, p. 353-358.
40. SUMMERS, WK., et al.: Oral tetrahydroaminoacridine in long-term treatment of senile dementia Alzheimer type. N. Engl. J. Med., 1986, vol. 315, p. 1241-1245.
41. SUSSMANN, JL., et al.: Atomic structure of acetylcholinesterase from Torpedo californica: A prototypic acetylcholine-binding protein. Science, 1991, vol. 253, p. 872-874.
42. UHLIG, M. - LUPPA, H.: Charakterisierung esterspaltender Enzyme des Gehirns mit Hilfe der Polyacrylamid-Gel--Elektrophorese. Acta histochem., 1975, Suppl. 15, p. 133-137.
43. VOLPE, MT. - BISSO, GM. - MICHALEK, H.: In vivo and in vitro effects of diisopropyl fluorophosphate and paraoxon on individual molecular forms of rats brain acetylcholinesterase. Neurochem. Res., 1990, vol. 15, p. 975-970.
44. WEISE, C., et al.: Anionic subsites of the acetylcholinesterase from Torpedo californica: Affinity labelling with the cationic reagent N,N-dimethyl-2-phenyl-aziridium. EMBO J., 1990, vol. 9, p. 3885-3888.
45. WHITTAKER, M.: Plasma cholinesterase variants and the anaesthesist. Anaesthesia, 1980, vol. 35, p. 174-197.

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