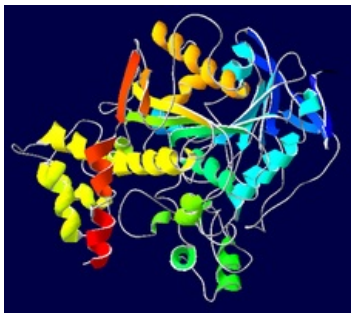
Cholinesterase

We distinguish between *acetylcholinesterase (ACHS)*, which selectively hydrolyzes acetylcholine, is produced at synapses of cholinergic nerves and in neuromuscular junctions, and is contained in erythrocytes; and between a related enzyme, sometimes referred as *pseudocholinesterase*, which also cleaves other choline esters. It is produced in the ribosomes of liver cells and is secreted into the bloodstream. A decrease in CHS activity in the blood serum is considered pathological. ACHS is sometimes measured in the amniotic fluid when looking for congenital hereditary defects.



Acetylcholinesterase

Testing

To test the activity of ACHS, the most frequently used substrate is *butyrylthiocholine iodide*, which is cleaved by ACHS into butyrate and *thiocholine iodide*, which further reacts with 5,5´-dithiobis-2-nitrobenzoic acid to form a colored substrate named 5-mercapto-2 nitrobenzoate, measurable at 410 nm; or **thiocholine iodide** reduces Fe^{2+} and the decrease in absorbance is measured at 405 nm. The test is performed on non-hemolytic serum or on blood plasma with an addition of heparin or EDTA. ACHS activity is stable in serum for up to 17 days at 4°C, and up to 3 months at −20°C.

Evaluation

Possible causes of the *reduction* of activity in the serum can be proteosynthesis disorder in severe hepatopathy, but also protein malnutrition (chronic starvation, tumor cachexia). The reduction can also be caused by intoxication with organophosphates, which are non-competitive inhibitors of this enzyme (chemical warfare agents, but also some insecticides). An inherited defect in cholinesterase synthesis is clinically silent in familial idiopathic acholinesterasemia, but there is a risk of an apneic pause due to cholinesterase deficiency after succinylcholine (a muscle relaxant) is given.

An *increase* in serum activity occurs, for example, in patients with increased proteosynthesis in nephrotic syndrome, in the recovery phase after hepatitis, in alcoholism, and in some other conditions. It has no diagnostic value. In patients with myasthenia gravis, autoantibodies directed against acetylcholine receptors are found in the serum; the symptoms of the disease are caused by an insufficient response to acetylcholine, i.e. muscle weakness to paresis. Among other things, cholinesterase inhibitors are used as the therapy for this condition.

ACHS physiological values at 37°C are **87-140 μkat/l for children up to 15 years**, **66.7-210.4 μkat/l for men** and **85.2–195.4 μkat/l for women**.

History

The existence of enzymes that can hydrolyze the neurotransmitter acetylcholine (ACh) was predicted in 1914 (Dale), before the actual discovery of the enzymes, as a result of an animal response to ACh administration. In 1926, a Calabar bean alkaloid (a poison that paralyzes the central nervous system, previously used in witchcraft), which was used in biochemistry as an inhibitor, has further demonstrated the existence of cholinesterase. In 1940, 2 main forms of ChE were discovered (Alles and Hawes). The two main closely related hydrolases from this family have been named acetylcholinesterases. Both enzymes are type B carboxypeptidases due to their biochemical properties. Both enzymes also exist in multiple molecular forms, both are ubiquitously distributed. AChE is more tissue specific than BChE. Both are sensitive to organic inhibition. In mammals, AChE and BChE have a strong primary homologous structure. Although the structure of ChEs has been studied for over 75 years, many characteristics are still unknown.

Cholinesterase and dementia treatment

In mild to moderate forms of dementia, cholinesterase inhibitors may be indicated, which is used in Alzheimer's disease therapy. Of course, there are patients who tolerate the treatment well and those who aren't responsive. Individual variability can be caused by the form of the disease, diagnostic inconsistencies, abnormalities in the metabolism of acetylcholine and other physical and mental comorbidities, or morphological changes in the CNS.

The principle of treatment is to affect the acetylcholinergic system by administering cholinesterase inhibitors, administering acetylcholine precursors, administering nicotinic and muscarinic agonists receptors, influencing the acetylcholinergic system by other mediators' pathways and some other mechanisms.

One of the most successful therapeutic methods is the application of cholinesterase inhibitors (IChE).

Side effects

The nature of the side effects varies depending on the stage of treatment in which the inhibitors are administered. During the titration phase of treatment (acute), the basic titration interval for IChE administration is four weeks. Titration is necessary with rivastigmine and galantamine in order to reduce the incidence of gastrointestinal side effects. Titration is not necessary for donepezil. Acute adverse effects and side effects are identical for the entire IChE group, therefore they are also referred as "class-effects". These effects are caused by an increased central cholinergic activity in the area postrema of the hypothalamus. Gastrointestinal side effects seem to be dominant in this phase. Side effects in this phase of treatment can be reduced, for example, by increasing the dose gradually (i.e. titration). If administered with food or shortly after eating, a significant reduction of possible adverse effects can be achieved. Male patients and patients with higher body mass tend to have a lower incidence of acute side effects. More serious stages of the disease are usually accompanied by a lower incidence of side effects. The increase occurs when titration is neglected, the medication administered on an empty stomach, in female patients, in milder stages of the disease and also in vascular dementia. The simultaneous administration of drugs blocking the hepatic metabolism of IChE can also be a potential risk.

Long-term treatment phase

At this stage, adverse and side effects differ for individual IChEs. The difference exists due to the fact, that, in addition to central cholinergic activity, the peripheral one is included as well. Blockade of individual cholinesterase isoforms, especially G1, G2 and G4, also plays a significant role in side effects variety. The individual isoforms differ in their chemical structure and topographical distribution in the CNS and PNS. The most clinically significant is the blockage of the G1 isoform. It is found in the cortex, hippocampus and amygdala, i.e. in those areas of the CNS that are the most important for Alzheimer's disease development. A massive blockage is therapeutically desirable, however, it results in a higher frequency of gastrointestinal side effects in the acute phase of treatment, especially when the dose is rapidly increased. G4 isoform blockage has a less specific therapeutic effect. Thanks to its higher occurrence in the medulla oblongata, brainstem and caudate nucleus, it can affect sleep and cause possible cardiovascular or extrapyramidal side effects. The G2 isoform is mainly found peripherally. Its impact on cognitive functions is relatively insignificant, although it can play role in the development of muscle-related side effects.

Pharmacokinetic and pharmacodynamic issues in cholinesterase inhibitors therapy

Patients with AD are mostly elderly and often present with other chronic diseases, mainly somatic ones. Drug interactions might be a frequent issue as well, as patients usually take a lot of medications with different pharmacological properties. E.g. donepezil may cause metabolic interactions, but has a suitably long half-life, thus can provide stable blood levels. Metabolic interactions are also reported with galantamine, it has a shorter half-life, hence fluctuating blood levels. CYP 450 inhibitors and inducers are used. During IChE therapy, cholinergic transmission is increased not only in clinically desirable areas, but also in other areas of the CNS and in certain peripheral organs. Bradyarrhythmia may occur in the cardiovascular system; nausea, dyspepsia and diarrhea in the gastrointestinal tract. Respiratory system can suffer from bronchoconstriction. Despite the abovementioned risks, IChE drugs are considered to be safe, however, treatment monitoring is important, as each patient will tolerate it differently.

Biological monitoring

Cholinesterase can be used in biological monitoring, which is a determination of the monitored toxic substance or its metabolites directly in the body. Urine, stool, exhaled air, hair or saliva can be used as samples. The determined substance is called an exposure biomarker. A toxic substance can disrupt normal biochemical processes in the body, which is manifested by a change in the levels of physiologically occurring substances in urine or blood. A perfect example is cholinesterase, which is responsible for the transmission of nerve impulses. If a person comes in contact with organophosphate insecticides, it leads to inhibition and a decrease in cholinesterase activity, which can be measured as an exposure biomarker.

Methods of analysis

Biological materials are often extracted, concentrated and converted into easier-analyzed forms. Samples for metal analysis are often mineralized prior to measurement. Chromatographic methods are used, mainly gas chromatography with a universal flame ionization detector, alternatively with a selective detector for nitrogenous substances or an electron capture detector for compounds with halogen atoms, and HPLC with a UV detector. AAS and mass spectrometry are used to determine metals.

Links

Related articles

Reference:

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