CAPSULES
notes and applications from Bioanalytical Systems, Inc.

Determination of Acetylcholinesterase Activity

Purpose

Determination of acetylcholinesterase (AChE) activity in brain tissue. AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) to choline (Ch) and acetic acid.

Perhaps the best studied transmitter substance is ACh. ACh is an excitatory transmitter released at some neuromuscular junctions, at some neuroglandular junctions, and at synapses between certain brain and spinal cord cells. ACh released by the stimulated presynaptic neuron binds to receptor sites on the postsynaptic neuron membrane and increases the permeability to Na^+ ions. This depolarizes the membrane and a nerve impulse is generated. The transmission of a continuous impulse is normally prevented by AChE, which is released into the synapse by the postsynaptic neurons. AChE hydrolyzes ACh to the neurochemically inactive compounds Ch and acetic acid. This permits the postsynaptic membrane to repolarize so that another impulse may be generated.

AChE is primarily localized in nerve cells associated with cholinergic synaptic transmission, but it is also found in a variety of other neuronal and non-neuronal cells. The determination of AChE activity is one of the essential biochemical indices used to evaluate the functional state of cholinergic neurons.

Existing Methods

Most commonly radiometric, colorimetric, fluorometric, and titrimetric assays have been used. Drawbacks of these methods include extensive sample handling, and lack of selectivity and sensitivity.

Reference

Highly Sensitive Assay for Acetylcholinesterase Activity by High-Performance Liquid Chromatography with Electrochemical Detection, N. Kaneda, Y. Noro, and T. Nagatsu, J. Chromatogr. 344(1985) 93-100. Dr. Nagatsu has been a scientific advisor to BAS Co., Ltd. in Tokyo. He is one of the premier neurochemists in the world and has contributed a number of useful LCEC assays.

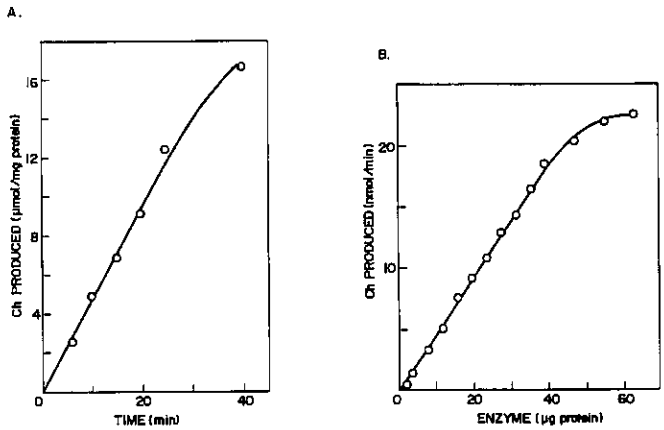

Figure 1. A) Rate of ACh hydrolysis by AChE from bovine caudate nucleus (3.94 µg protein added). B) AChE activity from bovine caudate nucleus as a function of enzyme (protein) concentration. Redrawn from above cited reference.

Conditions

Electrode: Platinum
Potential: +0.5 V vs Ag/AgCl
Column: 10 µm ODS, 250 x 4.6 mm (Yanagimoto, Japan)
Mobile Phase: 0.01 M sodium acetate-citrate (pH 5.0), containing 1.2 mM tetramethylammonium chloride, and 15 mg/L sodium octyl sulfate. Flow rate was 1.0 mL/min.
Post-column Addition: 0.2 M potassium phosphate (pH 8.5) was infused at 0.6 mL/min. This mixture, after passage through a Teflon coil, enters a

column containing immobilized choline oxidase.

Detection Limit: Sufficient to determine AChE activity in less than 0.1 mg of brain tissue.

Linear Range: 2 - 40 µg of protein of a brain homogenate.

Enzyme Assay

AChE activity in the brain homogenate was determined by incubating an aliquot under standard conditions with ACh as substrate and determining the amount of Ch produced. Ch is separated from the other reaction components (via LC) and enzymatically converted (post column) to H_2O_2 which is detected electrochemically on a platinum electrode.

Comments

This assay for AChE activity has a number of advantages: 1) it is highly sensitive--being comparable with that of most radiometric assays--requiring less than 0.1 mg of brain tissue; 2) it is a simple and specific--deproteininzed reaction mixture can be directly injected--LC conditions and a specific enzymatic reaction, with EC detection of the product, assures specificity; and 3) reproducibility is excellent--C.V. of 1.7% for Ch produced, n = 7 using the same enzyme preparation. It would be possible to study the changes in AChE activity in animal models of various diseases of cholinergic neurons.

Notes

1. Another enzyme, cholinesterase (ChE) can be present in various mammalian brain tissues. This enzyme is distinct from AChE, in that it more readily catalyzes the hydrolysis of butyrylcholine than acetylcholine. This enzyme is also referred to as butyryl cholinesterase (ButChE), pseudocholinesterase, or serum cholinesterase (or simply cholinesterase). Cholinesterase is also found in mammalian blood, plasma, liver, pancreas, and intestinal mucosa. AChE is found in red blood cells. AChE and ChE are two distinct enzymes.

ChE in rat brain did not utilize ACh as substrate. Therefore, the values for (AChE activity) obtained in this report (above cited reference) would not involve a contribution from ChE activity.

2. AChE inhibitors are of considerable importance, either for their therapeutic or toxic properties. The alkaloid physostigmine (eserine) and its related inhibitor neostigmine have been used to treat glaucoma and myasthenia gravis. Recent studies have indicated a role for physostigmine in the treatment of Alzheimer-type dementia. Physostigmine and neostigmine inhibit AChE by forming a covalent (carbamoyl-enzyme) intermediate that is hydrolyzed at a very slow rate, in contrast to the acetyl-enzyme intermediate (formed with ACh as substrate). Thus, the active site of AChE is effectively blocked. The therapeutic rationale is that these drugs inhibit AChE and thereby enhance the effects of endogenous ACh.

Organophosphates are even more potent inhibitors of AChE. These compounds react with AChE to form stable covalent phosphoryl-enzyme complexes. Many organic phosphate compounds have been produced for use as agricultural insecticides or as nerve gases. The EPA recommends periodic evaluation of AChE activity for pesticide applicators to determine whether pesticide exposure has occured.

Related References

1. LCEC Capsule No. 165 and 166 (determination of ACh and Ch).

2. LCEC Capsule 139 and 155 (assay for choline acetyltransferase (CAT) activity).

3. The results presented in this report can be duplicated utilizing a BAS Acetylcholine Analyzer (see product brochure for details). For those who wish to use the technique of immobilizing the enzymes on a post-column reactor (immobilized enzyme post-column reactor, IEPCR), BAS stocks an Acetylcholine/Choline Accessory Kit (BAS, P/N MF-8910). The kit consists of the analytical column, post-column reactor, platinum working electrode, enzymes, standards, and a detailed protocol for charging and utilizing the IEPCR for determination of ACh and CH in brain tissue. A practical application of the kit, using the new BAS 200 Liquid Chromatograph, can be found in the BAS journal, Current Separations, Vol. 7, No. 2.

One can readily see that both enzymatic procedures, homogeneous (enzymes in solution) and heterogeneous (enzymes immobilized on a solid phase), will serve the need for determining ACh-Ch, and AChE-CAT activity in a variety of samples. However, problems may arise with samples containing an anticholinesterase, at least in the case of the heterogeneous procedure. Given that the inhibitor migrates through the analytical column, it can bind to and inactivate the AChE immobilized in the IEPCR, drastically shortening its lifetime. Since fresh AChE is continuously infused in the homogeneous procedure, this situation should not cause a problem. However, the inhibitor may migrate with ACh and inhibit the soluble AChE, thus preventing the hydrolysis of ACh to Ch. The latter situation appears the least probable.

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