CAPSULES
notes and applications from Bioanalytical Systems, Inc.

Determination of Choline Acetyltransferase Activity

Purpose

Determination of choline acetyltransferase (CAT) activity in brain tissue. CAT catalyzes the synthesis of acetylcholine (ACh) from the immediate precursors choline (Ch) and acetyl-CoA.

CAT is recognized as a specific marker for cholinergic neurons and is thought to play an important role together with the action of acetylcholinesterase in regulating the concentration of ACh in the brain.

A reduction in CAT activity has been linked to senile dementia. Dementia is most commonly associated with Alzheimer's disease. Clinical symptoms of the disease are loss of memory and disorientation, followed by depression, hallucinations and finally death. Histologically the disease is characterized by the clumping of fibers within nerve cells (neurofibrillary tangles) and by the presence of senile plaques or amyloids (proteins with a fibrous structure). Diseased brain samples contain only 10-40% of the CAT activity compared to age-matched controls.

Existing Methods

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

Reference

Highly Sensitive Assay for Choline Acetyltransferase Activity by High-Performance Liquid Chromatography with Electrochemical Detection, N. Kaneda and T. Nagatsu, J. Chromatogr. 341(1985) 23-30. 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.

Conditions

Liquid Chromatography: LC-304T Electrochemical Analyzer.
Electrode: Pt, (BAS, P/N MF-1012).
Potential: +0.5 V vs. Ag/AgCl.
Column: 5 µm phenyl reverse-phase, 250 x 4.6 mm (Nomura Chemical, Japan)
Mobile Phase: 0.01 M sodium acetate-citrate (pH 5.0) containing 0.4 mM tetramethylammonium chloride and 30 ng/L sodium octyl sulfate.
Flow Rate: 0.8 mL/min.
Post Column Addition: 0.2 M potassium phosphate (pH 8.5) containing 2 U/mL acetylcholinesterase and 1 U/mL choline oxidase infused at 0.5 mL/min. The mixture of column effluent and enzyme solution reacts during passage through a Teflon coil.
Detection Limit: Sufficient to determine CAT activity in submilligram samples of brain tissue.
Linear Range: 16-128 µg of protein from the soluble fraction of brain homogenate.
Enzyme Assay: The enzyme solution consisted of the 20,000 x g soluble fraction of a brain tissue homogenate. CAT activity in the enzyme solution was determined by incubating an aliquot under standard conditions and determining the amount of ACh formed. ACh 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 CAT activity has a number of advantages: 1) it is highly sensitive -- the limit of sensitivity is 5 pmol of ACh synthesized; 2) it is simple and specific -- deproteinized reaction mixture can be directly injected -- LCEC and specific enzymatic reactions assure specificity; and 3) it is reproducible -- C.V. of 2.6% for ACh formed, n = 7 using same enzyme solution. It would be possible to study the

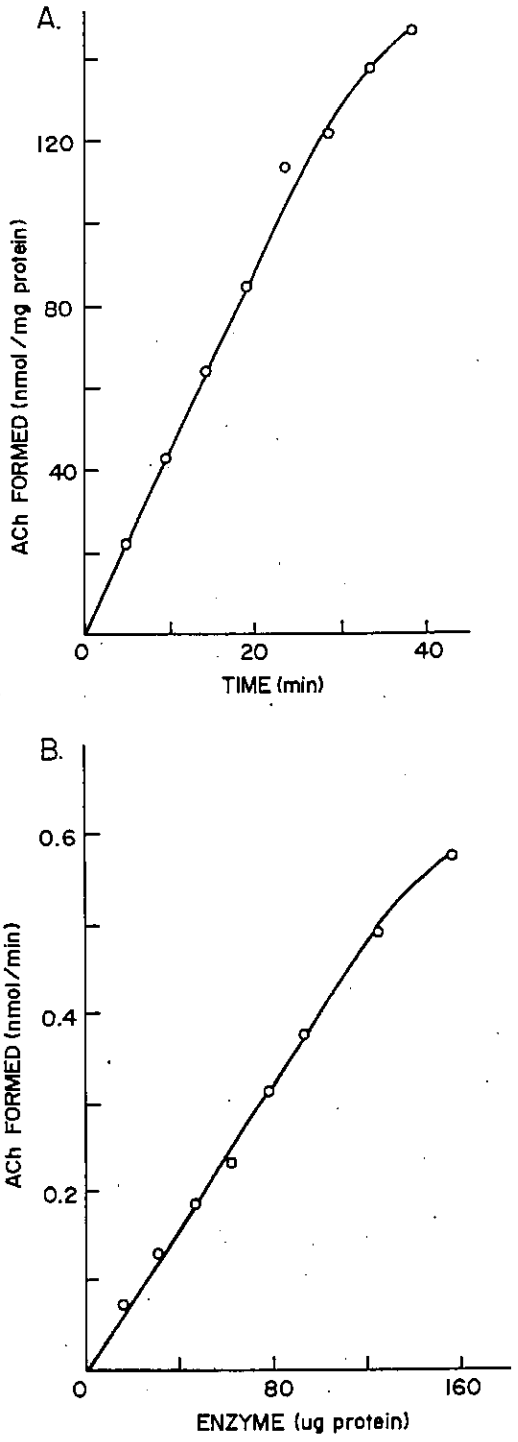

Figure 1. A) Rate of ACh synthesis by CAT from bovine caudate nucleus (64 µg protein added). B) CAT activity from caudate nucleus, as a function of enzyme (protein) concentration. Redrawn from above cited reference.

changes in CAT activity in animal models of various diseases, or in post mortem human brain samples.

Related References

1. *Capsule 165 and 166.*

2. *Capsule 139 and 142.*

3. *The results presented in this report can be duplicated utilizing a BAS 400 or BAS 200.*

The information in this publication is supplied as a service to our customers. Performance of the methodology has not necessarily been verified by the BAS technical staff.