Cholenirgic nerve terminals and senile plaques in Alzheimer's disease

1998 Fiscal Year Final Research Report Summary

Cholenirgic nerve terminals and senile plaques in Alzheimer's disease

Research Project

Project/Area Number

09670179

Research Category

Grant-in-Aid for Scientific Research (C)

Allocation Type

Single-year Grants

Section

一般

Research Field

Human pathology

Research Institution

Kanazawa University

Principal Investigator

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1997 – 1998

Keywords

vesicular acetylcholine transporter / glutathione-s-transferase / fusion protein

Research Abstract

Recently, vesicular acetylcholine transporter (VAChT), the proton-dependent transporter that packages acetylcholine synthesized in the cytoplasm into synaptic vesicles, has been known to be a good marker for morphological identification of cholinergic nerve terminals. In order to investigate how cholinergic nerve terminals are involved in formation of senile plaques in Alzheimer's disease, a specific antibody to human VAChT was tried to

be developed by using molecular genetics in this study. High molecular weight DNA was prepared from human blood cells and a genomic library was constructed in pWE cosmid vector after partial digestion of the genomic DNA with Mbo I.A library containing 1.3 X 10^6 independent colonies was screened with the human choline acetyltransferase (ChAT) cDNA obtained in the previous study, because human VAChT gene is located in the first intron of ChAT gene. Thirteen positive clones obtained were rescreened with human VAChT oligonuleotides probes reported, and then a genomic clone containing VAChT gene, Cos 25, was isolated. The full-length DNA of VAChT coding region or the DNA coding the carboxyl terminus was amplified by polymerase chain reaction and subcloned into an expression vector, pGEX-5X- 1. The protein containing glutathione-S-transferase fused with the VAChT carboxyl terminus was successfully expressed in E.coli bacterias. The fusion protein was purified from the bacterial lysates with the affinity matrix Glutathione Sepharose 4B.After further purification of the human VAChT carboxyl terminus with gel filtration for elimination of contaminated bacterial proteins, rats will be immunized for polyclonal antibody production with the human VAGhT carboxyl terminus.