Increased γ-Aminobutyrate Aminotransferase Activity in Brain of Patients with Alzheimer's Disease

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In order to search for more proximal factors in the pathogenesis of Alzheimer's disease, we studied the activities of various enzyme in the brains of patients, as well as control cases, by postmortem autopsy. In addition to the findings already known, such as the increase in prolyl endopeptidase (post-proline cleaving enzyme, PPCE) activity and the decrease in kallikrein activity, we found, anew, an increase in aminobutyrate aminotransferase (GABA-T) activity in the Alzheimer brain. This may be an important impetus for the reduction of γ-aminobutyric acid (GABA) in the brain, one of the neurotransmitters. It has to be determined whether the former two abnormalities offer a background for such an abnormality of the neurotransmitter.

Keywords γ-aminobutyrate aminotransferase; prolyl endopeptidase; kallikrein; Alzheimer's disease

Abnormal amyloid β-protein deposits are found in the brain of patients with Alzheimer's disease. Although the mechanism of the generation of such amyloid β-proteins is not entirely clear, many investigators believe that an imbalance between proteases and their inhibitors (α1-antichymotrypsin, *etc.*) in cerebral tissue underlies such events.[1–3] Furthermore, there is evidence indicating that protease inhibition by the longer chained amyloid β-protein precursor (ABPP) may be related to aberrant amyloid β-protein catabolism.[4] In line with these notions, we recently suggested that kallikrein deficiency in the brain is one of the important factors in the pathogenesis of the disease.[5] However, since it is not likely that the abnormalities is only a few specific enzymes can cause such extensive protein changes as mentioned above, we studied further looking for more factors which could possibly play major roles. This paper reports the abnormality in the Alzheimer brain of aminobutyrate aminotransferase (GABA-T), which is known to be a major inhibitory transmitter in the vertebrate central nervous system.[6]

Materials and Methods

Subjects Examined We examined 6 cases of senile dementia of the Alzheimer type (5 females, 84.8±3.5 years; 1 male, 91 years) and 6 control subjects (3 males, 88.7±5.7 years; 3 females, 72.3±1.3 years). Histologically, brains from these 6 cases with dementia had senile plaques and neurofibrillary tangles of various amounts, but no vascular lesions detectable by routine examination. Control subjects had neither clinical records of dementia nor pathologically significant lesions in the brains. The cerebral tissues were taken within several hours after death and were stored in a deep freezer at a temperature of −70 °C. The relation of enzyme activity to the postmortem hours was investigated in detail and was reported elsewhere.[5] In all cases the occipital lobe of the brain was used for enzymatic examination. The brain homogenates were prepared in phosphate-buffered saline (PBS, pH 7.2) by using a tissue homogenizer, Ultraturrax, at maximum speed for 1 min. The homogenate was centrifuged (3000 *g* for 20 min), and the supernatant fluid was withdrawn for measurement of enzymatic activity. The diagnosis was made according to pathological and clinical findings.

Substrates, Enzymes and Their Sources γ-[^{14}(U)]aminobutyric acid (GABA) for GABA-T, [^{14}C]putrescine, and [1-^{14}C]acetyl-coenzyme A (Ac-CoA) were obtained from New England Nuclear, Boston, U.S.A.; *N*,*N*-dimethylcasein for glutaminyl peptide γ-glutamyltransferase (transglutaminase, TG-ase), acetylcholine for acetylcholinesterase (AcCh-E), and choline bromide for choline acetyltransferase (ChAc-T) were obtained from Sigma Chemical Co., St. Louis, U.S.A.; carbobenzoxyarginyl-L-arginine β-naphthylamide (Z–Arg–Arg·NA) for cathepsin B, *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (NP–GlcNAc) for β-N-acetyl-D-glucosaminidase (GlcNAc-ase) and Z–Gly–Pro·NA for prolyl endopeptidase (PPCE) were from Bachem Feinchemikalien AG, Budendorf, Switzerland; L-prolyl-L-phenylalanyl-L-arginine-4·methylcoumaryl-7-amide (Pro–Phe–Arg·MCA) was from Peptide Institute Inc., Osaka, Japan.

Determination of Enzyme Activities The supernatant fluids of homogenates were dispensed into test tubes (1.5×10 cm) containing buffer to which the respective substrates were added. The test tubes were incubated for 1 h at 37 °C. The references for the assay methods and the substrates used are listed in Table I. All the enzyme assays in the supernatant fluids of homogenates were linear with time and enzyme concentration.[12] In the assay of kallikrein, the addition of bestatin at a dose of 200 to 400 μg/ml completely straightened the reaction curves.[5] All the enzyme assays were done in triplicate, and their standard deviations were within 10% of the average values.[5,12] The units of the enzyme activities were expressed as nmols of reaction products generated during one minute of incubation per mg protein (nmol/min/mg protein). Protein was determined by the method of Lowry *et al.*[15]

TABLE I. List of the Enzymes Measured and Their Substrates

Enzyme	Abbreviation	Substrate	Reference for assay method
Aminobutyrate aminotransferase (EC2.6.1.19)	GABA-T	GABA	7
Glutaminyl-peptide γ-glutamyltransferase (EC2.3.2.13)	TG-ase	Me-casein	8
Acetylcholinesterase (EC3.1.1.7)	AcCh-E	Ac-choline	9
Choline acetyltransferase (EC2.3.1.6)	ChAc-T	Choline	10
Cathepsin B (EC3.4.22.1)	Cathepsin B	Z–Arg–Arg·NA	11
β-*N*-Acetyl-D-glucosaminidase (EC3.2.1.30)	GlcNAc-ase	NP–GlcNAc	12
Tissue kallikrein (EC3.4.21.35)	Kallikrein	Pro–Phe–Arg·MCA	13
Prolyl endopeptidase (EC3.4.21.26)	PPCE	Z–Gly–Pro·NA	14

Abbreviations used: GABA, γ-aminobutyric acid; Z–Arg–Arg·NA, carbobenzoxyarginyl-L-arginine β-naphthylamide; NP–GlcNAc, *p*-nitrophenyl-*N*-acetyl β-D-glucosaminide; Pro–Phe–Arg·MCA, L-prolyl-L-phenylalanyl-L-arginine 4-methylcoumaryl-7-amide.

Results and Discussion

Table II compares the activities of various enzymes in the brain between control subjects and Alzheimer patients. The activities of GABA-T and PPCE were significantly increased, while that of kallikrein significantly decreased in the Alzheimer brain.

In this disease a reduction in brain contents of various neurotransmitters, such as acetylcholine, dopamine, norepinephrine, and GABA[16,17] has been reported. The present results show an increase in the GABA-T suggesting an increase in the breakdown of GABA, thus giving momentum for a reduction of GABA in the brain. Acetylcholinesterase (AcCh-E), another important enzyme for neurotransmission, did not show any significant changes.

In addition to these findings, we confirmed our previous reports which showed a decrease in the kallikrein activity and an increase in the activity of PPCE.[5] This kallikrein activity was suppressed by aprotinin, the specific inhibitor of kallikrein, as well as by leupeptin, but not by soy bean trypsin inhibitor (SBTI). Thus we concluded that the kallikrein activity found was not of plasma type but rather of tissue type.[5] As for PPCE, several investigators considered that its increased activity may be related to the decrease in vassopressin, which is the substrate of this enzyme and is said to facilitate the process of learning and memory.[18,19] It is not clear, at this point, whether all of these observations are associated with each other in the pathogenetic mechanisms of Alzheimer's disease. However, it seems reasonable to conclude that some fundamental abnormalities in the protease networks in the brain present a background for such abnormal metabolism including the precipitation of abnormal proteins as well as a decrease in neurotransmitters in the disease.

Finally, it is difficult to completely rule out the effects of pharmaceutical agents which had been given to the patients. Although we could not find any particular agents that are known to influence the enzymatic activities *in situ*, this will remain an important subject for our future studies.

TABLE II. Changes in Enzyme Activity in Brain of Patients with Alzheimer's Diseases

Enzyme	Specific activity (Mean ± S.D., nmol/min/mg protein)	
	Control ($n=6$)	Alzheimer ($n=6$)
GABA-T	0.036 ±0.025	0.183±0.144[a]
TG-ase	0.080 ±0.080	0.023±0.019
AcCh-E	0.14 ±0.03	0.34 ±0.29
ChAc-T	0.001 ±0.002	0.017±0.025
Cathepsin B	32.59 ±17.09	20.07 ±5.64
GlcNAc-ase	3.45 ±0.33	3.36 ±0.45
Kallikrein	0.0218±0.0059	0.014±0.005[a]
PPCE	7.47 ± 6.09	14.67 ±5.04[a]

a) $p<0.05$.

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