Increase in Aminobutyrate Aminotransferase and Cholineacetyltransferase in Cerebrum of Aged Rats

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We previously reported an increase in aminobutyrate aminotransferase (GABA-T) activity in the cerebrum of Alzheimer patients. In the present study, we investigated whether such findings are common in the usual aging process as well. We examined the activity of various enzymes, which were examined in the previous study, in the cerebrum of Wistar-Kyoto rats and spontaneously hypertensive rats. In the two strains, we compared the enzyme activities between the two groups of animals, 5 weeks and 13 weeks of age. In both strains, the older animals had significantly higher activities of GABA-T and choline acetyltransferase (ChAc-T). In spite of other enzymatic changes coexisting, the above two enzymes were suggested by discriminant function analysis to be playing a major role in the multiple enzymatic changes in the cerebrum of the animals.

Keywords aminobutyrate aminotransferase; cholineacetyltransferase; spontaneously hypertensive rat; rat cerebrum; age; neurotransmitter-related transferase

It has been reported that the cerebrum contents of various neurotransmitters, such as acetylcholine, dopamine, norepinephrine, and γ-aminobutyric acid (GABA), are reduced in Alzheimer's disease.[1,2] Consistent with such findings, our previous study demonstrated that the activities of aminobutylate aminotransferase (GABA-T) as well as prolyl endopeptidase (PPCE) in the cerebrum are significantly increased in the disease.[3,4] The present study was undertaken to investigate whether such findings are related to the aging process in general.

Materials and Methods

Experimental Animals Wistar-Kyoto rats (WKY) (male, 5 and 13 weeks of age) and spontaneously hypertensive rats (SHR) (male, 5 and 13 weeks of age) were obtained from Charles River Japan Inc., Atsugi, Kanagawa, Japan. They were kept on a pellet diet *ad libitum*. The animals were lightly anesthetized with ether and the systolic blood pressure was measured indirectly from the caudal artery and recorded with an automatic blood pressure recording device (model USM-105-R, Ueda Electric Works Ltd., Tokyo, Japan) by the tail-pulse-pickup method. Blood pressure in WKY was 105 ± 5 and 133 ± 5 mmHg at 5 weeks and 13 weeks of age, respectively, whereas in SHR it was 111 ± 6 and 181 ± 7 mmHg, respectively. The animals were killed by cervical dislocation and the cerebrum homogenates were prepared in phosphate-buffered saline (0.02 M, pH 7.2) using an Ultraturrax at the maximum speed for 1 min. The homogenate was centrifuged (5000 rpm, 20 min) and the supernatant fluid was withdrawn to measure enzyme activity.

Determination of Enzyme Activities The substrates, enzymes and their sources were as follows (see Table I for abbreviations): Glu·NA, Arg·NA, Pro·NA, Gly–Arg·NA, Lys–Ala·NA, Arg–Arg·NA, Gly–Pro·NA, Z–Gly–Pro·NA, Z–Arg–Arg·NA and NP–GlcNAc from Bachem Feinchemikalien AG, Budendorf, Switzerland; Pro–Phe–Arg·MCA, Boc–Gln–Ala–Arg·MCA and Suc–Ala–Pro–Ala·MCA from Peptide Institute Inc, Minoh-shi, Japan; GABA, acetylcholine and choline bromide from Sigma Chemical Co., St. Louis, U.S.A.; [1·^{14}C]acetyl-coenzyme A from New England Nuclear, Boston, U.S.A.

The supernatant fluids of homogenates were dispensed into test tubes 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.[5] All the enzyme assays were done in triplicate, and their

TABLE I. List of the Enzymes Measured and Their Substrates

Enzyme	Abbreviation	Substrate	Reference for assay method
Aspartate aminopeptidase (EC3.4.11.7)	AP-A	Glu·NA	5
Arginine aminopeptidase (EC3.4.11.6)	AP-B	Arg·NA	5
Proline iminopeptidase (EC3.4.11.5)	Pro-IP	Pro·NA	6
Dipeptidyl peptidase I (EC3.4.14.1)	DAP-I	Gly–Arg·NA	7
Dipeptidyl peptidase II (EC3.4.14.2)	DAP-II	Lys–Ala·NA	8
Dipeptidyl peptidase III (EC3.4.14.4)	DAP-III	Arg–Arg·NA	9
Dipeptidyl peptidase IV (EC3.4.14.5)	DAP-IV	Gly–Pro·NA	10
Prolyl endopeptidase (EC3.4.21.26)	PPCE	Z–Gly–Pro·NA	6
Cathepsin B (EC3.4.22.1)	Cathepsin B	Z–Arg–Arg·NA	11
Tissue kallikrein (EC3.4.21.35)	Kallikrein	Pro–Phe–Arg·MCA	12
Trypsin (EC3.4.21.4)	Trypsin	Boc–Gln–Ala–Arg·MCA	13
Elastase (EC3.4.21)	Elastase	Suc–Ala–Pro–Ala·MCA	14
Aminobutyrate aminotransferase (EC2.6.1.19)	GABA-T	GABA	15
Acetylcholinesterase (EC3.1.1.7)	AcCh-E	Ac–choline	16
Choline acetyltransferase (EC2.3.1.6)	ChAc-T	Choline	17
β-*N*-Acetyl-D-glucosaminidase (EC3.2.1.30)	GlcNAc-ase	NP–GlcNAc	5

Abbreviations used: Glu·NA, L-glutamic acid β-naphthylamide hydrochloride; Arg·NA, L-arginine β-naphthylamide hydrochloride; Pro·NA, L-proline β-naphthylamide hydrochloride; Gly–Arg·NA, glycyl-L-arginine β-naphthylamide; Lys–Ala·NA, L-lysyl-L-alanine β-naphthylamide; Arg–Arg·NA, L-arginyl-L-arginine β-naphthylamide; Gly–Pro·NA, glycyl-L-proline β-naphthylamide; Z–Gly–Pro·NA, carbobenzoxyglycyl-L-proline β-naphthylamide; Z–Arg–Arg·NA, carbobenzoxyarginyl-L-arginine β-naphthylamide; Pro–Phe–Arg·MCA, L-prolyl-L-phenylalanyl-L-arginine 4-methylcoumaryl-7-amide; Boc–Gln–Ala–Arg·MCA, *tert*-butyloxycarbonyl-L-glutaminyl-L-alanyl-L-arginine 4-methylcoumaryl-7-amide; Suc–Ala–Pro–Ala·MCA, succinyl-L-alanyl-L-prolyl-L-alanine 4-methylcoumaryl-7-amide; GABA, γ-aminobutyric acid; Ac–choline, acetylcholine; NP–GlcNAc, *p*-nitrophenyl-*N*-acetyl β-D-glucosaminide.

TABLE II. Age Dependent Changes in Enzymatic Activities in Cerebrum in SHR and WKY

	Specific activity ± S.D. (nmol/min/mg protein)			
	WKY		SHR	
	5W ($n=5$)	13W ($n=4$)	5W ($n=5$)	13W ($n=5$)
AP-A	0.77 ±0.06	0.70 ±0.10	0.98 ±0.13	1.10 ±0.45
AP-B	32.41 ±3.12	37.83 ±3.52[a]	47.24 ±2.35	51.85 ±5.15
Pro-IP	8.80 ±2.14	10.31 ±1.63	10.69 ±1.38	9.67 ±3.50
DAP-I	0.14 ±0.20	0.17 ±0.29	0.03 ±0.06	0.00 ±0.00
DAP-II	12.59 ±1.70	10.52 ±0.26	7.56 ±1.44	7.31 ±1.34
DAP-III	7.68 ±1.83	6.43 ±0.55	7.06 ±1.77	4.53 ±1.43[a]
DAP-IV	1.35 ±0.20	1.09 ±0.07[a]	1.27 ±0.21	1.02 ±0.16
Post-Pro-Enz	5.80 ±0.83	5.71 ±0.60	2.69 ±0.45	2.35 ±0.29
Cathepsin B	3.88 ±0.22	4.36 ±0.56	3.35 ±0.14	3.75 ±0.18[b]
Kallikrein	0.019 ±0.001	0.020 ±0.001	0.021 ±0.003	0.017 ±0.002[a]
Trypsin	0.031 ±0.001	0.027 ±0.006	0.042 ±0.005	0.030 ±0.006[a]
Elastase	0.100 ±0.009	0.099 ±0.010	0.121 ±0.007	0.098 ±0.005[c]
GABA-T	0.0052 ±0.0013	0.0101 ±0.0012[b]	0.005 ±0.001	0.006 ±0.007[a]
AcCh-E	1.02 ±0.08	1.02 ±0.30	1.46 ±0.54	1.46 ±0.26
ChAc-T	0.15 ±0.02	0.19 ±0.02[a]	0.17 ±0.04	0.27 ±0.03[b]
GlcNAc-ase	1.10 ±0.17	1.38 ±0.17[a]	1.48 ±0.20	1.33 ±0.15

a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$ (compared to 5W in Student's *t*-test). W = week.

standard deviations were within 10% of the average values.[3,5] For the assays, the units of enzyme activities were expressed as nmols of reaction products generated during one minute of incubation per milligram protein (nmol/min/mg protein). Protein was determined by the method of Lowry *et al.*[18]

Discriminant function analysis[19] is a method of multivariate analysis to find a linear combination of all variables measured that has the property of maximally separating the two groups of animals, younger and older. Its mathematical procedures were summarized elsewhere.[20]

Results and Discussion

In Table II are compared the activities of proteases and other enzymes in the cerebrum tissue between the animals 5 weeks and 13 weeks of age in WKY and SHR.

In WKY animals, the activities of AP-B, GABA-T, ChAc-T, and GlcNAc-ase were significantly increased in the aged group compared to the younger group, whereas those of DAP-IV decreased. In SHR, however, the activities of cathepsin B, GABA-T, and ChAc-T were increased, whereas those of DAP-III, kallikrein, trypsin, and elastase decreased with increase in age. Thus, comparing these enzymatic changes between the two strains of animals, common changes were seen only with regard to GABA-T and ChAc-T. Different behaviors of other enzymes between the two may somehow be related to the difference in the degree of organ lesions caused by hypertension.

Since the two transferase enzymes were increased in both strains, their roles in the multiple enzymes were tested by a multivariate analysis, discriminant function analysis. This function was used to maximally separate the young and the old groups in WKY and SHR, and the two groups could be completely differentiated without overlapping. In Table III are shown the correlations (called the structure coefficients) of the enzyme ativities to the discriminant function scores obtained for each animal.

The highest correlation was seen for GABA-T ($r=0.70$) but the correlation was similar to ChAc-T ($r=0.69$). Taken together with the above findings, these enzymes related to neurotransmitters seem to play major roles in the aging process. In our previous study the activity of GABA-T was increased (6-fold) in Alzheimer patients, while there was no significant change seen in the activity of ChAc-T.[4] This may mean that Alzheimer's disease is not a mere extension of the usual aging processes in the cerebrum.

TABLE III. Correlation of Discriminant Function Score to Various Enzymatic Activities

Enzyme	Correlation (r)	Enzyme	Correlation (r)
AP-A	0.08	Cathepsin B	0.49[a]
AP-B	0.31	Kallikrein	−0.28
Pro-IP	0.49[a]	Trypsin	−0.57[a]
DAP-I	−0.09	Elastase	−0.46[a]
DAP-II	−0.27	GABA-T	0.70[c]
DAP-III	−0.57[a]	AcCh-E	0.08
DAP-IV	−0.66[b]	ChAc-T	0.69[c]
PPCE	−0.13	GlcNAc-ase	0.16

a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

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