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Changes with Aging in the Levels of Amino Acids in Rat CNS Structural Elements II. Taurine and Small Neutral Amino Acids

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Taurine (Tau) and the small neutral amino acids glycine (Gly), serine (Ser), threonine (Thr), and alanine (Ala) were measured in 53 brain areas of 3- and 29-month-old male Fisher 344 rats. The ratio of highest to lowest level was 34 for Tau, 9.1 for Thr, 7.6 for Gly and Ser, and 6.5 for Ala. The heterogeneity was found in numerous areas; for example, Tau levels were more than 90 nmol/mg protein in 6 areas, and less than 20 nmol/mg protein in 10 areas. Similar heterogeneity was found with the other amino acids. The relative distribution of the small neutral amino acids showed several similarities; Tau distribution was different. With age, four amino acids decreased in 10-18 areas, and increased in only 1-3, while Thr increased in more areas than it decreased. The five amino acids of this paper, and the four of the previous paper, are among the amino acids at highest level in the brain; the sequence in their levels shows considerable regional heterogeneity.

KEY WORDS: Aging; taurine; neutral amino acids; amino acids.

INTRODUCTION

This paper is the second in our series examining the distribution of amino acids in distinct brain areas, with microdissection of 53 specific regions. The aim of the work, rather than to estimate distribution in whole brain and gross sections of the brain, is to analyze heterogeneity of amino acid levels in well-defined structures.

In the previous paper of this series we reported the distribution of aspartate, glutamate, glutamine, and GABA (1); in the present paper we report the distribution of five other amino acids, taurine, glycine, serine, threonine, and alanine, in the same structures. The four amino acids of the previous paper combined with the five of the present one are among those present at highest levels in brain of all species (average, 0.4-10 mM), with the other amino acids always being lower than these nine (average below 0.1 mM) (excluding a few compounds that are determined in amino acid analysis, but are usually not considered as amino acids such as cystathionine and phosphoethanolanine). The role of these amino acids in metabolism and as neurotransmitters also distinguishes them from most of the others. Of the five amino acids assayed in the present paper, serine and threonine may be used as glycine precursors, and alanine through its relationship to the Krebs cycle may have influence on glutamate formation; thus, each of them may have an important role in brain function in addition to that in protein synthesis.

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EXPERIMENTAL PROCEDURE

The animals assayed and the methods used were the same as described in the previous paper (1); this study presents further data on the distribution of amino acids in fifty-three microdissected brain areas. Briefly, 3-month-old and 29-month-old Fischer 344 male rats were used. The brain areas were obtained from coronal sections by the punch technique (2). The tissue samples were frozen, then homogenized, with cooling in perchloric acid. The protein content was determined by the micro Lowry method (3) on duplicate samples with a SD of 5-10% of the mean. The amino acids were assayed with precolumn derivatization (4) and reverse-phase high-pressure liquid chromatography. The sensitivity of this method is 5 pmol amino acid in the sample, and variation between repeat runs of the same sample was below 5 percent. The values in the tables are the averages of six tissue samples analyzed. Variation between samples (SD) was 4-8% of the mean, and repeat assay of the same sample gave values within 5% of the first assay.

RESULTS

The levels of the five amino acids assayed in this study, taurine, glycine, serine, threonine, and alanine, expressed as nmol of amino acid per mg of protein, are shown in five sets of brain areas: sensory (Table I), motor (Table II), hypothalamic (Table III), limbic (Table IV), and lower brain stem (Table V). The distribution of each of the five amino acids was heterogeneous, that of taurine more so than that of the other four. The concentration ratio of the highest level to the lowest is 34 for taurine, 9.1 for threonine, 7.6 for glycine or serine, and 6.5 for alanine. The concentration ratio of the averages of the three highest level areas to those of the three lowest level areas is 19 for taurine and 6-7 for the other amino acids (7.1 for glycine, 6.6 for serine, 5.8 for threonine, and 5.9 for alanine). This heterogeneity was not restricted to only a few nuclei: several areas in each case were very high and several were very low. With taurine 6 areas had over 90 nmol of amino acid per mg of protein and 10 had under 20, with glycine 6 areas had over 40 and 12 under 13, with serine 8 areas had over 27 and 7 under 8, with threonine 6 areas had over 14 and 4 under 4, and with alanine 6 areas had over 22 and 5 under 5.

When the six highest level areas of these amino acids are compared, there seems to be some relationship in the relative distribution of glycine, serine, threonine, and alanine. Four areas were among the highest with respect to all four amino acids: sensory trigeminal nucleus, medial preoptic nucleus, arcuate nucleus, and ventromedial nucleus. The distribution of taurine was different, however; for example, taurine levels were not high in any of these areas.

There was also some similarity among areas in which Gly, Ser, Thr, and Ala were low: in the cerebellar cortex, all four were among those at their lowest level; in the cingulate cortex and the medial septal nucleus, three of the four were near their lowest level and the fourth was also rather low; in the ventral horn, Ser, Thr, and Ala were near their lowest level, Tau was also very low, and only Gly was very high. There were a few dissimilarities: in the anterior hypothalamic nucleus, Ser was

Table I. Sensory Brain Areas

	nmol Amino Acid Per mg Protein									
	Taurine		Glycine		Serine		Threonine		Alanine	
Areas	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo
General Sensory										
Sensory cortex	52	43	7.6	7.5	19	15	6.2	7.3	8.5	8.6
Ventral thalamic nucleus	24	23	29	32	14	15	9.5	12	19	23
Sensory trigeminal nucleus	24	24	52	49	27	25	14	15	22	20
Nucleus gracilis	9.8	9.7	35	28	13	11	6.5	6.3	11	9.2
Nucleus cuneatus	18	20	45	51	13	15	5.8	8.3	10	14
Dorsal horn (spinal cord)	18	13	51	46	12	18	6.5	7.4	9.9	9.7
Special Sensory										
Visual cortex	110	96	25	19	19	18	8.6	9.9	18	14
Lateral geniculate body	34	36	20	18	14	13	6.5	7.5	7.6	8.0
Superior colliculus	29	28	37	29	19	15	12	10	16	12
Cochlear nuclei	26	16	42	30	19	22	9.5	12	15	16
Medial geniculate body	23	20	30	25	18	15	12	12	22	19
Inferior colliculus	27	24	38	34	22	21	16	18	17	16
Vestibular nuclei	20	18	28	26	14	14	7.5	7.0	11	10

For details see Methods section. Number of samples = 6
Standard deviation between 5 and 10%.

Table II. Motor Brain Areas

	nmol Amino Acid Per mg Protein									
	Taurine		Glycine		Serine		Threonine		Alanine	
Areas	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo
Pyramidal and Parapyramidal										
Motor cortex	42	39	9.5	10	20	18	5.3	6.8	7.4	8.3
Pontine nuclei	19	14	25	24	19	14	9.5	8.8	17	14
Ventral horn (spinal cord)	6.4	5.3	35	27	4.7	4.1	3.6	3.3	3.8	2.5
Extrapyramidal										
Caudate nucleus	80	70	7.9	7.6	16	17	7.0	7.5	9.2	9.3
Putamen	95	62	25	18	30	20	8.4	8.0	13	8.2
Globus pallidus	34	28	12	10	11	9.2	3.8	4.2	4.6	2.5
Substantia nigra	44	38	24	23	15	12	11	11	9.7	8.6
Cerebellar cortex	36	24	7.8	6.5	4.5	5.3	3.5	4.1	5.0	4.2
Cerebellar nuclei	63	51	32	27	13	9.7	9.5	11	8.3	6.1

For details see Methods section. Number of samples = 6
Standard deviation between 5 and 10%.

Table III. Hypothalamic Areas

	nmol Amino Acid Per mg Protein									
	Taurine		Glycine		Serine		Threonine		Alanine	
Areas	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo
Medial preoptic nucleus	37	35	58	43	31	27	14	16	22	19
Anterior hypothalamic nucleus	102	94	13	13	6.3	6.8	31	27	12	10
Supraoptic nucleus	25	27	16	15	23	31	9.8	13	15	23
Paraventricular nucleus	25	27	22	24	16	16	8.0	9.4	17	19
Arcuate nucleus	39	40	48	41	33	30	14	17	25	25
Median eminence	62	47	28	18	29	16	12	9.4	25	18
Ventromedial nucleus	47	26	54	36	34	19	17	10	23	15
Dorsomedial nucleus	20	18	17	17	17	18	7.0	9.9	14	14
Lateral hypothalamus (MFB)	34	27	30	24	24	18	13	11	18	14
Posterior hypothalamic nucleus	13	12	26	24	7.4	8.8	5.4	6.5	7.3	9.1

For details see Methods section. Number of samples = 6
Standard deviation between 5 and 10%

very low and Thr was very high. Again, taurine was not at low levels in areas where the other four were low.

The four amino acids assayed in the first paper of this series, glutamate, glutamine, aspartate, and GABA and the ones assayed in the present paper are at high level in all parts of the brain. If the six highest level amino acids are examined in each area, only seven amino acids were in each case among the six highest - Glu, Gln, GABA, Asp, Tau, Gly, and Ser - but the sequence varied among the areas assayed (Table VI). If amino acids at the highest level are examined, Glu was the highest in 23, GABA in 16, Asp in 5, Tau in 5, and Gln in 4 areas. The amino acid that was the 6th highest in sequence was also variable: Tau was the 6th in 10 areas, Gly in 17, Ser in 13, Glu in 2, GABA in 1, and Asp in 1 area (Table VI). Ser was never higher than 6th among the amino acids, but the others were more variable. Ser was the 6th highest amino acid in 13 areas; in one of these it displaced Tau, in the other 12 it displaced Gly, from the top 6. Thr and Ala were not among the 6 highest level amino acids in any of the areas, and with very few exceptions were below Ser and Gly levels, as also found in previous studies in whole brain in a number of species (5).

The changes with age are shown in Tables VII and VIII. If significant changes are compared, four amino acids (Tau, Gly, Ser, Ala) show mostly decreases, one more (Thr) increases. Tau decreased in 21 areas, Gly decreased in 22 and increased in 1, Ser decreased in 13 and increased in 2, and Ala decreased in 22 and in-

Table IV. Limbic Brain Areas

	nmol Amino Acid Per mg Protein									
	Taurine		Glycine		Serine		Threonine		Alanine	
Areas	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo
Olfactory bulb	215	202	11	12	20	22	12	14	18	20
Olfactory tubercle	91	78	12	13	27	27	9.8	12	11	11
Cingulate cortex	39	41	7.1	9.3	7.9	9.5	3.4	4.6	9.4	10.4
Pyriform cortex	69	61	18	13	27	24	7	8	16	11
Hippocampus	57	41	7.3	4.5	11	8.0	4.9	4.8	9.5	9.1
Dentate gyrus	118	108	29	26	20	18	8.9	10	18	16
Medial septal nucleus	32	22	10	7.6	9.0	7.8	3.6	3.8	4.7	3.6
Lateral septal nucleus	42	37	16	16	13	13	5.7	6.7	11	12
Medial amygdaloid nucleus	49	44	14	9.9	22	21	7.0	8.2	12	11
Lateral amygdaloid nucleus	46	42	13	16	16	17	5.0	5.8	9.7	9.1
Central amygdaloid nucleus	42	34	16	9.8	20	15	6.4	5.6	10	8.6
Bed nucleus, stria terminalis	35	29	16	17	12	12	5.6	6.8	9.9	10
Habenula	45	40	30	23	20	16	9.4	8.7	15	12
Interpeduncular nucleus	28	24	20	19	18	14	8.6	6.7	12	7.8

For details see Methods section. Number of samples = 6
Standard deviation between 5 and 10%.

Table V. Lower Brain Stem Areas

	nmol Amino Acid Per mg Protein									
	Taurine		Glycine		Serine		Threonine		Alanine	
Areas	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo	3 mo	29 mo
Periaqueductal central gray	25	22	36	30	12	11	9.7	9.5	11	8.5
Dorsal raphe nucleus	18	16	24	20	11	9.3	8.0	9.1	12	8.6
Parabrachial nuclei	12	12	31	27	9.8	8.3	6.1	6.7	9.5	8.1
Locus coeruleus	31	29	21	23	11	9.1	9.4	10	14	11
Reticular formation	13	12	33	32	7.9	7.3	6.7	6.5	7.2	5.8
Nucleus of the solitary tract	14	14	37	28	14	11	7.0	6.3	11	8.3
Central gray (spinal cord)	7.0	5.3	35	27	5.7	4.8	3.7	3.7	4.0	3.8

For details see Methods section. Number of samples = 6
Standard deviation between 5 and 10%.

creased in 3. In contrast, Thr increased in 7 and decreased in 2 areas.

DISCUSSION

The amino acids examined in the present paper, along with those of the previous paper, are present at high level in all species, and their relative level (glutamate is usually higher than aspartate, which is higher than alanine) is also similar in the brain of various species examined. In comparing amino acid levels in various species (6-7) taurine seems to be the most variable, being 2-4 times higher in rat and mouse brain than in brain of other species, such as rabbit, guinea pig, cat, dog, monkey, and man. Most amino acids are lower in frog brain (7).

The regional distribution of amino acids has been studied in a number of laboratories in the past. The studies that analyzed all the amino acids utilized large brain areas, composed of many CNS anatomical subdivisions, while the studies utilizing a larger number of areas measured only a few amino acids. Our studies are thus novel in two respects, that we measured all of the amino acids in a large number (53) of anatomically well-defined brain structures, and that in addition, we compared adult and aging brain values. In an earlier study (8) we analyzed all of the amino acids, by dividing the whole brain into four sections; even among such large areas significant heterogeneity was found, with a 5.8 ratio of highest to

Table VI. The Amino Acids at the Highest Levels in the Areas Examined

	1.	2.	3.	4.	5.	6.
SENSORY AREAS						
Sensory cortex	Glu	Gln	Tau	Asp	GABA	Ser
Ventral thalamic nucleus	Glu	Gln	Asp	GABA	Gly	Tau
Sensory trigeminal nucleus	Asp	Glu	Gly	Gln	GABA	Tau
Nucleus gracilis	Gln	Glu	Gly	Asp	GABA	Ser
Nucleus cuneatus	Gln	Gly	Glu	Asp	GABA	Tau
Dorsal horn (spinal cord)	Glu	Gly	Gln	Asp	GABA	Tau
Visual cortex	Glu	Tau	Gln	GABA	Asp	Gly
Lateral geniculate body	Glu	Gln	Asp	GABA	Tau	Gly
Superior colliculus	GABA	Gln	Asp	Glu	Gly	Tau
Cochlear nuclei	Glu	Asp	Gly	Gln	Tau	GABA
Medial geniculate body	Asp	Gln	Glu	GABA	Gly	Tau
Inferior colliculus	Asp	GABA	Gly	Gln	Glu	Tau
Vestibular nuclei	GABA	Gln	Glu	Asp	Gly	Tau
MOTOR AREAS						
Motor cortex	Glu	Tau	Gln	Asp	GABA	Ser
Pontine nuclei	Asp	Glu	Gln	Gly	GABA	Tau
Ventral horn (spinal cord)	Glu	Asp	Gly	Gln	GABA	Tau
Caudate nucleus	Tau	Glu	Gln	GABA	Asp	Ser
Putamen	Glu	Tau	Gln	GABA	Asp	Ser
Globus pallidus	GABA	Tau	Glu	Gln	Asp	Gly
Substantia nigra	GABA	Asp	Gln	Tau	Glu	Gly
Cerebellar cortex	Glu	Gln	Tau	Asp	GABA	Gly
Cerebellar nuclei	Tau	GABA	Glu	Asp	Gln	Gly
HYPOTHALAMIC AREAS						
Medial preoptic nucleus	GABA	Asp	Gln	Gly	Glu	Tau
Anterior hypothalamic nucleus	GABA	Tau	Glu	Asp	Gln	Gly
Supraoptic nucleus	Asp	GABA	Glu	Gln	Tau	Ser
Paraventricular nucleus	GABA	Gln	Asp	Glu	Tau	Gly
Arcuate nucleus	Gln	GABA	Asp	Glu	Gly	Tau
Median eminence	Glu	Gln	Tau	Asp	GABA	Gly
Ventromedial nucleus	GABA	Asp	Gln	Gly	Tau	Glu
Dorsomedial nucleus	GABA	Gln	Asp	Glu	Tau	Gly
Lateral hypothalamus (MFB)	GABA	Asp	Gln	Tau	Gly	Glu
Posterior hypothalamic nucleus	Gln	GABA	Glu	Asp	Gly	Tau
LIMBIC AREAS						
Olfactory bulb	Tau	GABA	Glu	Gln	Asp	Ser
Olfactory tubercle	Tau	Glu	GABA	Gln	Asp	Ser
Cingulate cortex	Glu	Tau	GABA	Asp	Gln	Ser
Pyriform cortex	Glu	Tau	Gln	Asp	GABA	Ser
Hippocampus	Glu	Tau	GABA	Gln	Asp	Ser
Dentate gyrus	Tau	Glu	Gln	GABA	Gly	Asp
Medial septal nucleus	Glu	Asp	GABA	Tau	Gln	Gly
Lateral septal nucleus	GABA	Glu	Gln	Tau	Asp	Gly
Medial amygdaloid nucleus	Glu	Gln	GABA	Tau	Asp	Ser
Lateral amygdaloid nucleus	Glu	Gln	GABA	Tau	Asp	Ser
Central amygdaloid nucleus	GABA	Glu	Gln	Tau	Asp	Gly
Bed nucleus, stria terminalis	GABA	Glu	Gln	Tau	Asp	Gly
Habenula	Glu	GABA	Asp	Gln	Tau	Gly
Interpeduncular nucleus	GABA	Asp	Glu	Gln	Tau	Gly
LOWER BRAIN STEM AREAS						
Periaqueductal central gray	GABA	Glu	Gln	Asp	Gly	Tau
Dorsal raphe nucleus	GABA	Glu	Gln	Asp	Gly	Tau
Parabrachial nuclei	Glu	GABA	Gln	Asp	Gly	Tau
Locus coeruleus	Glu	Gln	Asp	GABA	Tau	Gly
Reticular formation	Glu	Asp	GABA	Gly	Gln	Tau
Nucleus of the solitary tract	Glu	Gln	Gly	GABA	Asp	Tau
Central gray (spinal cord)	Glu	Gln	Gly	Asp	GABA	Tau

The six highest amino acids are shown in sequence of level 1 = highest.

lowest for glycine and 3.9 for taurine. Another study (9) of four brain areas (hemisphere, midbrain, cerebellum, and pons medulla) found most ratios to be below 2. A more recent analysis of five areas (brain stem, hypo-

Table VII. Percent Change in Amino Acid Levels in Old Rats (Motor and Sensory Areas)

	Tau	Gly	Ser	Thr	Ala		Tau	Gly	Ser	Thr	Ala
SENSORY AREAS						MOTOR AREAS					
Sensory cortex	-17^{d}	0	-21^{a}	$+18^{a}$	0	Motor cortex	0	0	0	$+28^{2}$	$+12^{a}$
Ventral thalamic nucleus	0	0	0	$+26^{3}$	$+21^{b}$	Pontine nuclei	-26^{c}	0	-26^{1}	0	-18^{b}
Sensory trigeminal nucleus	0	0	0	0	0	Ventral horn (spinal cord)	-17^{c}	-23^{2}	-13^{9}	0	-34^{c}
Nucleus gracilis	0	-20^{a}	-15^{a}	0	-16^{a}	Caudate nucleus	-13^{b}	0	0	0	0
Nucleus cuneatus	$+11^{a}$	$+13^{a}$	$+15^{a}$	$+43^{b}$	$+40^{b}$	Putamen	-35^{d}	-28^{d}	-33^{d}	0	-37^{d}
Dorsal horn (spinal cord)	-28^{a}	0	$+50^{a}$	$+14^{c}$	0	Globus pallidus	-18^{d}	-17^{a}	-16^{a}	$+11^{a}$	-45^{a}
Visual cortex	-13^{c}	-24^{c}	0	$+15^{a}$	-22^{e}	Substantia nigra	-14^{a}	0	-20^{a}	0	-11^{a}
Lateral geniculate body	0	0	0	$+15^{a}$	0	Cerebellar cortex	-33^{e}	-17^{a}	$+18^{b}$	$+17^{a}$	-16^{b}
Superior colliculus	0	-22^{d}	-21^{b}	-17^{a}	-25^{d}	Cerebellar nuclei	-19^{e}	-16^{d}	-26^{e}	$+16^{a}$	-27^{b}
Cochlear nuclei	-38^{e}	-29^{d}	$+16^{a}$	$+26^{a}$	0						
Medial geniculate body	-13^{a}	-17^{c}	-17^{a}	0	-14^{a}						
Inferior colliculus	-11^{a}	-11^{b}	0	$+13^{a}$	0						
Vestibular nuclei	0	0	0	0	0						

^{a} = nonsignificant
^{b} = $P < 0.05 - P < 0.02$
^{c} = $P < 0.02 - P < 0.01$
^{d} = $P < 0.01 - P < 0.001$
^{e} = $P < 0.001$

Table VIII. Percent Change in Amino Acid Levels in Old Rats in Hypothalamic, Limbic, and Lower Brain Stem Areas

	Tau	Gly	Ser	Thr	Ala		Tau	Gly	Ser	Thr	Ala
HYPOTHALAMIC AREAS						LIMBIC AREAS					
Medial preoptic nucleus	0	-26^{a}	-13^{a}	$+14^{a}$	-14^{c}	Olfactory bulb	0	0	0	$+17^{a}$	$+11^{a}$
Anterior hypothalamic nucleus	0	0	0	-13^{d}	-17^{a}	Olfactory tubercle	-14^{a}	0	0	$+22^{a}$	0
Supraoptic nucleus	0	0	$+35^{e}$	$+33^{b}$	$+53^{c}$	Cingulate cortex	0	$+31^{b}$	$+20^{a}$	$+35^{a}$	$+11^{a}$
Paraventricular nucleus	0	0	0	$+18^{a}$	$+12^{a}$	Pyriform cortex	-12^{b}	-28^{a}	-11^{a}	$+14^{a}$	-31^{d}
Arcuate nucleus	0	-15^{9}	0	$+21^{a}$	0	Hippocampus	-28^{e}	-38^{e}	-27^{e}	0	0
Median eminence	-24^{a}	-36^{d}	-44^{d}	-22^{a}	-28^{b}	Dentate gyrus	0	0	0	$+12^{a}$	-11^{b}
Ventromedial nucleus	-45^{d}	-33^{d}	-44^{e}	-41^{d}	-35^{d}	Medial septal nucleus	-31^{e}	-24^{b}	-13^{b}	0	-23^{d}
Dorsomedial nucleus	0	0	0	$+41^{e}$	0	Lateral septal nucleus	-12^{b}	0	0	$+18^{a}$	0
Lateral hypothalamus (MFB)	-21^{b}	-20^{c}	-25^{b}	-15^{a}	-22^{c}	Medial amygdaloid nucleus	0	-29^{c}	0	$+17^{a}$	0
Posterior hypothalamic nucleus	0	0	$+19^{a}$	$+20^{a}$	$+25^{a}$	Lateral amygdaloid nucleus	0	$+23^{a}$	0	$+16^{a}$	0
LOWER BRAIN STEM						Central amygdaloid nucleus	-19^{b}	-39^{b}	-25^{c}	-13^{a}	-14^{a}
Periaqueductal central gray	-12^{a}	-16^{b}	0	0	-23^{b}	Bed nucleus, stria terminalis	-17^{b}	0	0	$+21^{d}$	0
Dorsal raphe nucleus	-11^{a}	-17^{d}	-15^{a}	$+14^{a}$	-28^{b}	Habenula	-11^{b}	-23^{d}	-20^{a}	0	-20^{e}
Parabrachial nuclei	0	-13^{a}	-15^{a}	0	-15^{b}	Interpeduncular nucleus	-14^{b}	0	-22^{a}	-22^{1}	-35^{e}
Locus coeruleus	0	0	-17^{d}	0	-21^{c}						
Reticular formation	0	0	0	0	-19^{c}						
Nucleus of the solitary tract	0	-24^{d}	-21^{b}	0	-25^{c}						
Central gray (spinal cord)	-24^{b}	-23^{d}	-16^{b}	0	0						

Symbols for significance are the same as in Table VII

thalamus, hippocampus, corpus striatum, frontal cortex) found much more pronounced regional heterogeneity, with 20- to 30-fold differences between the highest and lowest content levels of aspartate, glutamate, glutamine, and GABA, and 60-fold differences for taurine (10); that those values are higher than the ones reported in this paper is due primarily to the very low levels of all amino acids found in the brain stem region in the former study. Our early study comparing brain stem with hemisphere, midbrain, and cerebellum (8) found that some amino

acids (Tau, Glu, GABA) are low in brain stem but others (Ala, Asp, Ser, Gly) are not, and the heterogeneity of such gross areas is moderate.

A review of results of four laboratories found a cortex-to-pons medulla concentration ratio for taurine of approximately 3 in each study (11). In an analysis of 25 regions of postmortem human brain, the highest to lowest ratios were GABA 5.3, Asp 3.1, Tau 2.7, Ala 2.1, and Glu 2.1 (12). Our present study of the rat demonstrates highest to lowest ratios of 34, 9.1, 7.6, 7.6, and 6.5 for Tau, Thr, Gly, Ser, and Ala, respectively. A somewhat lower degree of heterogeneity was found by Palkovits et al. (13) in analyzing taurine distribution, and Elekes et al. (14) in measuring the distribution of glycine and serine. The latter two studies, like our present one, analyzed a large number (44) of well-defined structures in Wistar rats.

Although there is a large variation in amino acid levels of individual brain nuclei, a certain dominance of particular amino acids in major brain areas can be observed: GABA content is the highest in 6 nuclei of the hypothalamus; aspartate level is high in the acoustic system (highest in the medial geniculate and the inferior colliculus, the second highest in the cochlear nuclei); glutamate is the highest in several lower brain stem nuclei; and taurine levels are very high in the limbic lobe (highest in the olfactory bulb and tubercle and in the dentate gyrus, and the second highest in the pyriform cortex and the hippocampus).

Since an uneven regional distribution of an amino acid may indicate another functional role in addition to that in protein synthesis, several studies examined the distribution of neurotransmitter amino acids in the spinal cord. In a recent study, the distribution of Gly, GABA, Asp, Ala, and Glu was measured in micropunch samples from nine cord areas (15). GABA distribution showed high heterogeneity (highest to lowest ratio, 3.5), and the other amino acids were distributed fairly homogeneously.

Although the functional significance of relative distribution and quantitative rank order of the amino acids is not clear at present, some indication for function can be derived from studies where distribution or rank order was altered. Several studies examined alterations in amino acid distribution in pathological conditions and alterations caused by drugs. Some studies examined possible changes in excitatory or inhibitory amino acids in epileptic foci. It was suggested by van Gelder (16) that an imbalance of Tau, Glu, and Gln is best correlated with seizures. Apparently seizures can occur in the absence of taurine deficiency, and there also can be an absence of seizures in the presence of taurine deficiency (17). The increase in glycine upon lithium treatment may participate in the pharmacological action of lithium (18). Such drug effects can be region and amino acid specific. Amphetamine was shown to increase Asp only, phenylethylamine Tau only, with no effects on Glu, Ala, or GABA (19). In the cerebellum but not in other brain areas of the staggerer mouse mutant, Glu was lower while Gly and Ala were higher than in controls; in the weaver mutant, GABA and Gly in the brain stem were higher (20). Decreased Glu, Asp, and GABA were found in some areas of postmortem Alzheimer brains (21). Acute stress reduced GABA and increased Gly levels significantly, but only in a few brain areas (14). These changes indicate that the control of levels is also amino acid and region specific, and as a consequence the alterations of distribution are also amino acid and region specific, and when particular cells and subcellular elements are considered, possibly even more specific than revealed in the past studies.

In assessing the heterogeneity of amino acid distribution, it is important to realize that significant differences may exist in the level of an amino acid between cells and between subcellular elements, and are especially likely with the neurotransmitter amino acids. Studies on transport of GABA show differences between neuronal and glial uptake (22, 23), suggesting differences in control of, and in pharmacological or physiological responses of, GABA levels in different cell types. Some differences may exist in taurine uptake between astrocytes and granule cells (24). Neuronal release and glial removal represent a complex metabolic cycle, including glia-neuron exchange and neuronal sequestration. Vesicle-specific neurotransmitter translocators with possibly different properties (ion requirements) and higher substrate specificity have been shown to exist for many amino acids, such as specific transport systems for GABA, Asp, and Glu, discussed in previous papers (25, 26). A similar system of high specificity for glycine has been shown to exist with a 10-fold higher transport activity in the spinal cord than in the cerebrum, possibly reflecting its functional role as a spinal cord transmitter (27). These and other studies indicate that levels at nerve endings are different from those in other structures (28) and are under different regulatory mechanisms.

Age-related changes in amino acid levels of brain nuclei show large variations. Significant depletion of all five amino acids investigated was measured only in hypothalamic (median eminence, ventromedial nucleus, lateral hypothalamus) and limbic (medial septal nucleus, central amygdaloid nucleus) areas. Especially low values (the average depletion was over 40%) were found in the ventromedial nucleus. There was only one brain nucleus,

the cuneate nucleus, where the level of five amino acids was higher in aging rats than in 3-month-old animals.

Taurine levels in the spinal cord were decreased (dorsal horn 25%, ventral horn 17%, central gray 23%) in old rats. Similarly, marked depletions were found in the septo-hippocampal system (medial septal nucleus 32%, hippocampus 29%) and in the cerebellum (cortex 35%, nuclei 19%).

The decrease in amino acids in some nuclei raises the possibility that these areas are more susceptible to age-related changes or that losses in other components may have preceded the decrease in amino acids. A correlation of amino acid changes with other changes with age may help in our evaluation of the changes found in the present study.

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