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Levels of amino acids in 52 discrete areas of postmortem brain of adult and aged humans

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Summary. In a series of studies we have analyzed the regional distribution of the free amino acid pool in 52 discrete areas of postmortem brain of adult and aged humans. Here we show the distribution of eleven amino acids: alanine, methionine, valine, leucine, isoleucine, glutamine, asparagine, lysine, arginine, ornithine, and histidine. As found previously for other amino acids, the distribution of these amino acids was seen to be heterogeneous, the level of the area of highest level being 3.4 to 10.7 times that of the area of the lowest level. On average we found a five- or six-fold difference in concentration between the highest and lowest level areas in the brain samples from adult and old respectively. The distribution patterns were found to be different for each amino acid; they were not similar even in the same class (amides, branched chain, basic amino acids), and they were different from those recently found in rat brain. Only a few changes, mostly increases, were found in the aged brain, such as increases in alanine and valine levels in cortical areas. In studies of changes in cerebral amino acid levels, the great regional heterogeneity of distribution has to be taken into account since changes in whole brain values may not reflect regional changes. The functional significance and the control of this regional heterogeneity are under investigation.

Keywords: Amino acids – Regional distribution – Aged brain – Amino acid levels – Human brain

Introduction

We began to study the distribution of free amino acids in large areas of brains of various species more than 20 years ago (Levi et al., 1967, 1968; Battistin et al., 1969; Battistin and Lajtha, 1970). Subsequent work further analyzed regional

differences in humans (Perry et al., 1971a; Perry, 1982). More recent work examined selected regions (Palkovits et al., 1986a,b; Donzanti and Ung, 1989; Jensen et al., 1991). These studies clearly showed significant regional heterogeneity in the distribution in the brain of all the amino acids assayed.

We felt that these studies, still assaying the larger areas, may have underestimated the heterogeneity that would have been revealed if much smaller areas had been analyzed. A set of such studies in rat brain (Banay-Schwartz et al., 1989a,b, 1990a,b) indeed indicated considerably larger differences in amino acid levels between well-defined nuclei, and prompted us to reexamine similar regions in human brain areas. The present paper, the third in a series examining amino acid levels in human brain regions, describes the distribution of amino acids not covered in the previous reports (Banay-Schwartz et al., 1992b, 1993).

Methods

Brain tissue was removed from the skulls of victims of traffic accidents, heart attack, or suicide. The subjects died unexpectedly. Interviews with family members did not indicate prior evidence of chronic disease. None were hospitalized or received chronic medical treatment in the months preceding their death. They had no pathological evidence of cerebrovascular disease, although the aged brains had a degree of sclerosis of major cerebral arteries. The traffic accident victims died before reaching a hospital and were therefore taken directly to the pathology lab. Only brain that had not been damaged and had been removed from the skull within 2 h of death was selected. The tissue either was dissected immediately after removal or was frozen on dry ice and kept at $-70°C$ until dissection. After being warmed to about $-10°C$, brains were cut into 2 to 3-mm thick coronal slices and brain regions were dissected from the slice on a cooled tray ($-10°C$) with special microdissection needles (inside diameters 1.5–8.0 mm). The forebrain and the diencephalon were sliced in the coronal plane; in the lower brain stem axis, sections were made perpendicular to the axis. Nuclei were removed from sections of approximately 1-mm thickness (Palkovits, 1973). Areas were identified under a stereomicroscope. Only a portion of each area was removed to avoid contamination by neighboring areas. All subjects were male – the six adult men had been 35, 38, 44, 46, 52, and 55 years old; the five old men, 71, 77, 78, 79, and 81.

The assay of amino acids was done by reverse-phase high-pressure liquid chromatography after precolumn derivatization, as described earlier (Banay-Schwartz et al., 1989a; Neidle et al., 1989). The frozen brain tissue samples were weighed, homogenized in 3% w/v perchloric acid, and left standing for 30 min at 0°C; part was used for protein determination, another part was centrifuged and the supernatant was neutralized. Amino acids were reacted with naphthylisocyanate, the excess reagent was removed with cyclohexane, and the amino acids were separated on a C18 silica column with a Shimadzu automated chromatography system, using one ultraviolet and one fluorescence detector, with norleucine as the internal standard. The sensitivity was below 5 pmol, and the variations, when a sample was analyzed several times, were between 2 and 5 percent. The variation among samples from different brains was greater. Some tissue samples were too small to weigh precisely – these are marked as **1** in the tables. Their weights were estimated from protein determinations, assuming an 8.5% protein content. The protein content of the 52 brain areas studied here was published previously (Banay-Schwartz et al., 1992a). The content of other amino acids in these samples, of glutamate and aspartate (Banay-Schwartz et al., 1992b), and of GABA, glycine, taurine, serine, threonine, phenylalanine, and tyrosine (Banay-Schwartz et al., 1993) have also been published.

In each of the tables the amino acid levels are expressed as μmol of amino acid per g of fresh tissue. The standard error of the values was mostly less than 8%, and the results are

the averages of six adult and five old brain samples analyzed individually, several in duplicate. In the tables, when values for old brains are significantly different from adult values they are marked * for $p < 0.01$, and ** for $p < 0.05$. Statistical analysis was performed using Student's t test for unpaired samples.

Results

The distribution pattern differed among the amino acids. Five amino acids are presented in Tables 1–4 – the small neutral amino acids alanine and methionine and the branched-chain amino acids valine, leucine, and isoleucine. In the sensory areas (Table 1) alanine was at its lowest level in the cochlear nuclei, while in most other areas it was at severalfold higher levels. The levels of the other amino acids were not particularly low in this area, except as will be shown later, that of histidine. In cortex some increase with age could be seen with alanine and valine. Valine, leucine, isoleucine, and alanine levels were fairly high in the superior colliculus; these amino acids and methionine were also at high levels in the posteromedial thalamic nuclei.

In the motor areas (Table 2), methionine and the branched chain amino acids were at low level in the cortex. Alanine, valine, and leucine reached their peak levels, of all the areas analyzed, in the inferior olive, an area fairly rich in the other amino acids as well. An increase in levels with age could be seen in the cortex.

In the limbic system (Table 3) methionine was low, while the other amino acids were at average levels, and were fairly evenly distributed. Methionine and the branched-chain amino acids were at low levels in white matter: valine was at its lowest in cerebellar white matter, but alanine not as low; values were not low in the cervical spinal cord. The increases for valine with age in the cortical areas were in the somatosensory cortex 113, occipital cortex 64, somatomotor cortex 90, frontal cortex 162, cingulate cortex 82 percent. The increases for alanine in these areas were in the somatosensory cortex 43, occipital cortex 44, somatomotor cortex 68, frontal cortex 70, and cingulate cortex 35 percent.

In the autonomic centers (Table 4), alanine and isoleucine were at peak levels in the central grey matter; the reticular formation was also rich in these amino acids (but not in the subsequent six presented in later tables). In the endocrine system, the preoptic area was rich in amino acids. Valine levels, but not leucine or isoleucine levels, were low in the supraoptic nucleus and the median eminence.

We also measured in the same human brain areas the distribution of the amino acid amides glutamine and asparagine, and that of the basic amino acids lysine, arginine, ornithine, and histidine. In the sensory areas (Table 5), glutamine reached its peak in the superior colliculus and posteromedial thalamic nuclei; asparagine levels were not particularly high in these areas. Lysine and arginine levels were similarly high in the ventral thalamic nuclei, but their levels differed in the superior and inferior colliculus, where arginine levels, but not lysine levels, were high. The distribution of these amino acids was parallel in several motor areas (Table 6). In the cortex, most amino acids were present at low levels (the lowest, asparagine and lysine), while levels in the red nucleus were high; only a

Table 1. Distribution in the sensory areas

	Alanine		Methionine		Valine		Leucine		Isoleucine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Somatosensory cortex	1.03	1.47**	0.11	0.10	0.30	0.64*	0.30	0.35	0.15	0.22
Occipital cortex	1.00	1.44*	0.08	0.12	0.25	0.41*	0.40	0.38	0.13	0.20
Ventral thalamic nuclei	2.18	1.93	0.16	0.14	0.58	0.56	0.75	0.84	0.32	0.38
Posteromedial thalamic nuclei	2.09	2.64	0.21	**0.24**	0.58	0.66	0.77	0.93	0.35	0.44
Pulvinar	1.92	1.84	0.14	0.11	0.50	0.35	0.71	0.99	0.35	0.43
Lateral geniculate body	1.70	1.42	0.20	0.19	0.50	0.53	0.72	0.58	0.35	0.30
Medial geniculate body	1.52	1.78	0.17	0.24	0.51	0.64	0.77	0.84	0.29	0.34
Superior colliculus	2.30	2.55	0.15	0.21	0.58	0.75	0.81	0.92	0.41	**0.64**
Inferior colliculus	0.62	0.57	0.14	0.16	0.41	0.44	0.64	0.66	0.29	0.28
Vestibular nuclei	1.63	1.54	0.12	0.09	0.45	0.41	0.64	0.59	0.27	0.25
Cochlear nuclei	**0.38**	**0.27**	0.15	0.17	0.35	0.47	0.51	0.50	0.20	0.26
Sensory trigeminal nucleus	2.16	2.60	0.20	0.20	0.54	0.60	0.68	0.83	0.35	0.47
Gracile nucleus[1]	1.51	1.54	**0.24**	0.20	0.60	0.60	0.64	0.77	0.37	0.37
Cuneate nucleus[1]	1.53	1.00	0.18	0.16	0.38	0.45	0.50	0.40	0.23	**0.15**

[1] estimated from protein content (see Methods)

* $p < 0.01$

** $p < 0.05$

Table 2. Distribution in the motor areas

	Alanine		Methionine		Valine		Leucine		Isoleucine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Somatomotor cortex	0.91	1.53*	0.09	0.09	0.20	0.38**	0.24	**0.29**	0.12	0.22**
Frontal cortex	0.80	1.36*	**0.06**	0.09	0.26	0.68*	**0.19**	0.33**	**0.10**	0.17**
Caudate nucleus	1.06	1.44	0.12	0.14	0.47	0.53	0.43	0.57	0.23	0.27
Putamen	1.37	1.57	0.15	0.19	0.44	0.57	0.55	0.80	0.28	0.34
Inner pallidum	1.13	1.02	0.12	0.14	0.18	0.27	0.46	0.37	0.22	0.21
Outer pallidum	1.37	1.46	0.12	0.10	0.44	0.44	0.47	0.38	0.22	0.23
Red nucleus	1.61	1.41	0.18	0.22	0.54	0.61	0.67	0.88	0.33	0.29
Substantia nigra	1.66	1.62	0.15	0.22	0.50	0.62	0.65	0.86	0.30	0.32
Pontine nuclei	1.24	1.23	0.16	0.18	0.50	0.42	0.53	0.51	0.28	0.23
Cerebellar cortex	1.45	1.31	0.14	0.16	0.40	0.42	0.64	0.63	0.24	0.28
Cerebellar nuclei	1.02	1.16	0.21	0.22	0.51	0.55	0.75	0.70	0.31	0.30
Inferior olive	**2.43**	**2.90****	0.22	0.22	**0.81**	**0.95**	**1.03**	**1.29**	0.27	0.26

* $p < 0.01$
** $p < 0.05$

Table 3. Distribution in the limbic system and in white matter

	Alanine		Methionine		Valine		Leucine		Isoleucine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Cingulate cortex	1.2	1.62**	0.09	0.10	0.33	0.60*	0.46	0.53	0.16	0.25
Septum	1.34	1.30	0.09	0.08	0.32	0.38	0.39	0.46	0.21	0.22
Lateral hypothalamus	1.49	2.11**	0.14	0.19	0.42	0.52	0.47	0.55	0.26	0.27
Mamillary body	1.65	1.90	0.15	0.14	0.47	0.38	0.69	0.64	0.35	0.32
Amygdala	1.86	2.06	0.13	0.16	0.45	0.63	0.50	0.70	0.27	0.32
Hippocampus	1.66	1.98	0.13	0.15	0.40	0.53	0.56	0.62	0.26	0.31
Anterior thalamic nuclei	1.90	2.08	0.19	0.14	0.51	0.46	0.61	0.73	0.28	0.36
Habenula	1.66	1.94	0.15	0.19	0.40	0.53	0.57	0.62	0.25	0.29
Parahippocampal cortex	1.17	1.27	0.12	0.11	0.36	0.42	0.46	0.50	0.24	**0.25**
Spinal cord (cervical)	1.43	1.73	0.11	0.09*	0.54	0.57	0.74	0.83	0.31	0.40
Cerebellar white matter	0.84	1.13	0.10	0.08	**0.15**	**0.12**	0.40	0.31	0.20	0.18
Cerebral white matter	0.78	1.10*	0.08	**0.07**	0.17	0.15	0.28	0.28	0.14	0.19*

* $p < 0.01$
** $p < 0.05$

Table 4. Distribution in the autonomic centers and the endocrine system

	Alanine		Methionine		Valine		Leucine		Isoleucine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Central grey matter	**2.35**	**2.63**	0.16	0.17	0.46	0.48	0.70	0.77	**0.42**	0.60
Dorsal raphe nucleus	1.74	2.08	0.12	0.14	0.40	0.39	0.58	0.62	0.23	0.42
Locus coeruleus	1.19	1.48	0.12	0.11	0.43	0.35	0.49	0.43	0.24	0.30
Tegmentum pontis	1.19	1.21	0.12	0.09	0.40	0.26	0.62	0.85	0.25	0.32
Ventrolateral medulls oblongata[1]	1.37	1.87**	0.20	0.20	0.40	0.34	0.50	0.60	0.20	0.20
Reticular formation	2.05	2.38	0.21	0.23	0.48	0.54	0.69	0.68	0.30	0.39
Dorsal vagal complex[1]	1.99	1.41**	0.16	0.10	0.50	0.46	0.60	0.61	0.27	0.30
Preoptic area	2.15	2.41	0.12	0.11	0.70	0.56	0.58	0.74	0.35	0.34
Supraoptic nucleus	1.54	0.96	0.10	0.14	0.28	0.22	0.51	0.35	0.25	0.19
Paraventricular nucleus[1]	1.51	1.06	0.16	0.14	0.55	0.44	0.71	0.79	0.24	0.26
Anterior hypothalamus	1.67	1.16	0.16	0.16	0.52	0.55	0.54	0.72	0.27	0.40
Stalk-median eminence	1.36	1.11	0.17	0.12**	0.23	0.23	0.49	0.68	0.21	0.28
Medial hypothalamus	1.32	1.22	0.13	0.15	0.44	0.49	0.59	0.64	0.22	0.29
Posterior hypothalamus	1.58	1.76	0.16	0.16	0.36	0.37	0.54	0.50	0.28	0.26

[1] see legend Table 1
* $p < 0.01$
** $p < 0.05$

Table 5. Distribution in the sensory areas

	Glutamine		Asparagine		Lysine		Arginine		Ornithine		Histidine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Somatosensory cortex	5.43	6.00	0.12	0.16	0.22	0.25	0.25	0.30	0.19	0.17	0.17	0.20
Occipital cortex	5.36	4.95	0.13	0.20	0.16	0.21	0.25	0.35	0.20	0.17	0.20	0.19
Ventral thalamic nuclei	6.97	6.85	0.35	0.41	**0.40**	0.36	**0.89**	0.68	0.18	0.14	0.19	0.15
Posteromedial thalamic nuclei	**12.2**	12.5	0.34	0.34	0.20	0.16	0.68	0.79	0.15	0.10	0.14	0.18
Pulvinar	10.9	12.2	0.33	0.29	0.27	0.28	0.60	0.45	0.24	0.20	0.26	0.36
Lateral geniculate body	7.46	6.37	0.31	0.25	0.32	0.18	0.56	0.38	0.25	0.16	0.23	0.21
Medial geniculate body	5.95	6.00	0.28	0.31	0.29	0.26	0.46	0.46	0.22	0.16	0.22	0.22
Superior colliculus	12.1	**15.0**	0.30	0.36	0.24	0.19	0.64	0.83	0.23	0.18	0.18	0.14
Inferior colliculus	5.48	5.72	0.35	0.30	0.23	0.26	0.64	0.85	0.17	0.14	0.16	0.16
Vestibular nuclei	4.56	6.02	0.37	0.36	0.26	0.25	0.49	0.60	0.16	0.14	0.17	0.18
Cochlear nuclei	5.92	8.76*	0.35	0.44	0.25	0.40	0.51	0.65	0.15	0.17	0.10	**0.10**
Sensory trigeminal nucleus	8.83	9.65	0.35	0.28	0.31	0.25	0.63	0.55	0.19	0.15	0.15	0.17
Gracile nucleus[1]	5.00	3.70**	**0.51**	0.57	0.24	0.25	0.47	0.60	0.20	0.19	0.19	0.14
Cuneate nucleus[1]	4.96	3.54	0.50	0.50	0.19	0.16	0.35	0.29	0.24	0.20	0.23	0.16

[1] see legend Table 1

* $p < 0.01$

** $p < 0.05$

Table 6. Distribution in the motor areas

	Glutamine		Asparagine		Lysine		Arginine		Ornithine		Histidine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Somatomotor cortex	4.41	5.45	**0.12**	0.15	0.17	0.17	0.27	0.38	0.13	0.20*	0.10	0.17
Frontal cortex	4.52	5.08	**0.12**	0.13	**0.09**	0.24**	0.29	0.60*	0.13	0.21*	0.13	0.18
Caudate nucleus	5.77	5.80	0.34	0.20	0.19	0.27	0.28	0.27	0.17	0.16	0.28	0.30
Putamen	6.32	6.40	0.27	0.22	0.30	0.25	0.50	0.45	0.20	0.15	0.26	0.37
Inner pallidum	7.40	7.36	0.38	0.41			0.24	0.22			0.12	0.22
Outer pallidum	6.48	8.28	0.46	0.49			0.27	0.18			**0.07**	0.11
Red nucleus	10.0	11.5	0.28	0.31	**0.40**	**0.48**	0.51	0.51	**0.28**	0.24	0.24	0.26
Substantia nigra	6.20	6.92	0.38	0.36	0.34	0.40	0.52	0.48	0.27	0.20	0.24	0.19
Pontine nuclei	7.97	9.38	0.35	0.26	0.22	0.21	0.31	0.32	0.21	0.17	0.32	0.24
Cerebellar cortex	5.52	7.86*	0.32	0.37	0.26	0.18	0.45	0.38	0.17	0.12	0.22	0.17
Cerebellar nuclei	6.74	7.39	0.31	0.31	0.39	0.27	0.57	0.57	0.22	0.17	0.21	0.17
Inferior olive	7.58	8.57	0.38	0.43	0.33	0.26	0.78	0.94	0.21	0.19	0.29	0.16

* $p < 0.01$
** $p < 0.05$

few changes (increases) in the cortex areas of the old were found. The basic amino acids lysine, arginine, and ornithine were increased in the aged frontal cortex.

In the limbic system (Table 7), in the septum and lateral hypothalamus the levels of lysine and ornithine, but not of arginine, were low. Levels of asparagine, but not of other amino acids, were low in the cingulate cortex. Asparagine levels were lower in most cortical areas. Amino acid levels were low in the white matter, especially in cerebral white matter.

In the autonomic centers (Table 8), glutamine was high in the dorsal raphe nucleus, while histidine levels were low in this area. In the endocrine system glutamine levels were low, lowest in the median eminence, and asparagine and histidine levels were highest in the paraventricular nucleus. Arginine and ornithine levels were high in the preoptic area.

In the cortical areas a number of amino acids showed increase with age (alanine, valine, isoleucine, arginine, and ornithine). It is of interest that while isoleucine shows an increase, leucine decreased in some of these regions. Glutamine tends to increase in several areas with age (vestibular and cochlear nuclei, cerebellum cortex, mamillary body), and histidine also shows increases in several areas.

Discussion

The present results, and those of previous papers assaying brain regions in human tissue (Banay-Schwartz et al., 1992b, 1993) and in rat tissue (Banay-Schwartz et al., 1989a,b, 1990a,b), show a significant heterogeneity in amino acid distribution. The highest average level is at least 5-fold higher than the lowest level, and the levels in the majority of the areas vary by 100% or more. The pattern of distribution is different in the various areas, and at present we do not understand the control or the function of such heterogeneity. It is not likely that the distribution pattern is a reflection of the distribution of amino acid transport systems. If one looks at the distribution of amino acids of the same transport system, such as the basic amino acids lysine and arginine or the large neutral branched chain amino acids valine, leucine, and isoleucine, the regional distribution patterns are not identical, indicating that transport activity, at least as measured by cellular uptake in brain slices or transport at the blood-brain interface, is not the only control factor. Other possible contributions could come from additional transport systems, since as has been shown, several amino acids have affinity to more than one system. Since more than 10 different transport systems for amino acids exist in the brain (Sershen and Lajtha, 1979), affinity to more than one system may present a complex picture. Additional complexity may be due to differences in mediated exit, the properties of which could be different from those of uptake. We previously failed to see a close relationship of tissue levels to uptake when measuring tissue uptake in slices of brain, in whole brain, or in brain areas (Kandera et al., 1968). Clearly, transport must play an important role, but additional processes – metabolic rates of amino acids, production of amino acids from proteins, transport compartments and metabolic compartments of amino acids – may also influence regional distribution.

Table 7. Distribution in the limbic system and in white matter

	Glutamine		Asparagine		Lysine		Arginine		Ornithine		Histidine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Cingulate cortex	5.60	5.70	0.17	0.19	0.18	0.27*	0.34	0.44	0.24	0.22	0.21	0.18
Septum	6.26	5.25	0.22	0.17	0.12	0.11	0.36	0.31	**0.08**	0.10	0.14	0.15
Lateral hypothalamus	10.5	10.4	0.28	0.27	**0.09**	**0.08**	0.38	0.35	0.09	**0.08**	0.11	0.11
Mamillary body	7.26	9.81	0.31	0.24	0.11	0.12	0.40	0.32	0.10	0.10	0.16	0.12
Amygdala	5.55	6.56*	0.24	0.27	0.21	0.21	0.41	0.34	0.22	0.14**	0.20	0.25
Hippocampus	6.43	6.44	0.28	0.29	0.21	0.18	0.45	0.43	0.24	0.14**	0.18	0.24
Anterior thalamic nuclei	11.7	10.9	0.35	0.27			0.69	0.72			0.15	0.11
Habenula	8.47	9.32	0.27	0.24	0.11	0.14	0.46	0.56	0.09	0.11	0.11	0.17**
Parahippocampal cortex	6.80	6.20	0.37	0.39	0.29	0.25	0.32	0.29	0.27	0.24	0.10	0.13
Spinal cord (Cervical)	5.66	4.55	0.37	0.40	0.19	0.25	0.24	**0.15**	0.16	0.14	0.17	0.23
Cerebellar white matter	3.76	3.84	0.25	0.15	0.15	0.20	0.22	0.26	0.11	0.14		
Cerebral white matter	4.70	5.00	0.16	**0.11**	0.12	0.15*	**0.19**	0.16	0.10	0.12*		

* $p < 0.01$
** $p < 0.05$

Table 8. Distribution in the autonomic centers and the endocrine system

	Glutamine		Asparagine		Lysine		Arginine		Ornithine		Histidine	
	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old	Adult	Old
Central grey matter	9.92	11.3	0.30	0.28	0.26	0.30	0.66	0.59	0.23	0.17	0.16	0.22
Dorsal raphe nucleus	10.9	13.4	0.29	0.26	0.25	0.30	0.23	0.26	0.22	0.25	0.13	**0.10**
Locus coeruleus	9.78	9.46	0.26	0.20	0.27	0.28	0.30	0.37	0.20	0.23	0.10	0.14
Tegmentum pontis	5.82	7.48*	0.30	0.32	0.21	0.26	0.48	0.66	0.18	0.19	0.18	0.14
Ventrolateral medulla oblongata[1]	7.17	8.4	0.30	0.30	0.14	0.11	0.32	0.27	0.20	0.10	0.16	0.19
Reticular formation	9.23	10.4	0.30	0.30	0.24	0.12	0.47	0.41	0.16	0.09	0.14	0.15
Dorsal vagal complex[1]	5.58	5.5	0.44	0.40			0.62	0.71			0.15	0.14
Preoptic area	7.20	5.75	0.34	0.41	0.18	0.16	0.76	**1.0**	0.21	**0.27**	0.2	0.23
Supraoptic nucleus	5.57	4.26	0.30	0.24	0.22	0.24	0.45	0.36**	0.17	0.16	0.20	0.19
Paraventricular nucleus[1]	6.19	6.12	0.50	**0.60**			0.51	0.71			**0.38**	**0.37**
Anterior hypothalamus	4.73	3.85	0.35	0.43**	0.33	0.24	0.33	0.32	0.16	0.16	0.13	0.13
Stalk-median eminence	**2.37**	**2.12**	0.30	0.35	0.18	**0.22**	0.22	0.47	0.10	0.10	0.13	0.13
Medial hypothalamus	3.95	3.72	0.42	0.47	0.21	0.34	0.37	0.41	0.14	0.16	0.14	0.13
Posterior hypothalamus	6.93	5.35	0.28	0.19	0.12	0.17	0.32	0.30	0.12	0.12	0.12	0.11

[1] see legend Table 1
* $p < 0.01$
** $p < 0.05$

It is at present difficult to estimate the consequences of heterogeneous distribution. One important aspect could be the formation of neurotransmitters from their amino acid precursors, since it has been shown that norepinephrine, dopamine, and serotonin synthesis rates are influenced by cerebral phenylalanine, tyrosine, and tryptophan levels (Hughes and Johnson, 1976, 1977). The formation of other metabolic products and amino transfer may also show regional heterogeneity as a consequence of heterogeneous levels in various structures.

The assay of small areas showed a greater heterogeneity in distribution than did that of larger areas. It seems likely that assay of yet smaller structures will reveal considerably greater differences in level, for example, when levels in single cells, synaptic vesicles, mitochondria, etc., are compared. Levels could differ by one or even two orders of magnitude. In order to avoid contamination by neighboring areas, we sampled only a portion of each structure, and we can not predict what the levels would be in the tissue not analyzed.

There may be several processes involving amino acids that are not sensitive to their levels. One example may be protein synthesis. We found that cerebral protein synthesis rates were not altered when amino acid levels were increased severalfold (Dunlop et al., 1975).

Necessarily, to obtain representative areas from each brain, postmortem tissue was used. Even though tissue was obtained within 2 hours of death, some changes in amino acid levels could have occurred. We did not find major postmortem changes in rodents (mice, rats, guinea pigs) when the intact brain was kept for various time periods (Lajtha and Toth, 1974), but in human brain differences between biopsied and autopsied tissue have been noted (Perry et al., 1971b, 1981). It is unlikely that regional heterogeneity is greatly influenced by postmortem changes.

The changes in level and in distribution in the aging brain are in most instances not large. This contrasts with the large changes found during development (Lajtha and Toth, 1973; Huether and Lajtha, 1991). Numerous changes in pathological tissue have been noted. Changes connected with seizures have been examined in detail (van Gelder et al., 1983; Simler et al., 1990; Lehmann, 1989; Hunter et al., 1989), and changes in numerous neurological pathologies have been reported (Perry et al., 1987, 1988; Guilarte, 1989). Changes with stress (Palkovits et al., 1986; Elekes et al., 1986), and by drugs that are in therapeutic use, were also reported (Toth and Lajtha, 1981; Perry et al., 1989; Sershen et al., 1982).

In the above and other previous studies of the pathological or drug-induced changes in cerebral amino acid levels, in most cases gross brain areas or whole brain tissue were analyzed, and there is little information available about regional variations in the changes; for example, whether the changes depend on regional levels, so that areas with high amino acid levels show proportionally greater changes than areas with lower levels.

The significant regional heterogeneity of levels indicates the need for reevaluation of the influences – to study changes according to the region, since the changes may be very different in different structures, even occurring in opposite directions, and may reinforce or change regional heterogeneity of

amino acid distribution. It is likely that cerebral amino acid levels undergo considerable change even under physiological conditions – for example, the postprandial increases of amino acids in the blood that alter brain uptake. Thus one could question the physiological significance of two-fold increases in the level of an amino acid when under physiological circumstances areas differ severalfold in levels; such changes may have no effect on brain function. Further work is needed to establish the significance of the heterogeneous distribution of amino acids, and of any changes in this distribution.

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