phy chemically related? What is the mechanism of ACh sensitivity induction - is it due to the increased activity of phagocytic cells[10] invading the region of EDL muscle where the proteins are released from plates, or is there a direct effect on the muscle fibre membrane?

The present results are in good accordance with the recent observation on muscle tissue cultures by Oh[11,12] who reports on a neurotrophic factor (probably a glycoprotein), capable of substituting for innervation and extractable from nerve tissue.

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Free amino acids in motor cortex of amyotrophic lateral sclerosis[1]

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Summary. Free amino acids were estimated quantitatively in the motor cortex from 3 patients with amyotrophic lateral sclerosis (ALS) and 11 control subjects. Among 7 amino acids which showed statistically significant changes, taurine was the only one which was increased constantly and most markedly in the motor cortex of all the 3 ALS cases. It was suggested that the metabolism of sulfur amino acids might be affected in comparatively early stages of ALS.

The pathogenesis of amyotrophic lateral sclerosis (ALS) is unknown. Although biochemical changes reported so far in nervous tissue of ALS might be mostly secondary phenomena, approaches along this line seem to be needed in order to find essential metabolic derangements of ALS.

Diverse changes in free amino acids in the ALS spinal cord[4] prompted us to examine the motor cortex in which histopathological changes were milder, in a sense, earlier, when compared to those seen in the spinal cord. It was found that the increase in taurine content which had been observed in the ALS spinal cord[4] was also a common change in the motor cortex of 3 ALS patients obtained at autopsy. The results of case 1 (a 52-year-old woman) have been reported previously[5].

Subjects and methods. 3 ALS patients autopsied were a 52-year-old woman, a 62-year-old woman and a 66-year-old man. They were all diagnosed as the classical, nonhereditary type of ALS by clinical and pathological findings. The total course of the illness was about 2, 1.5 and 2.5 years, respectively. The elapsed time between death and autopsy was about 3, 2 and 2.5 h, respectively. 11 control subjects had all died of nonneurological diseases, such as congestive heart failure, pneumonia, myocardial infarction, gastric ulcer, uterine myoma and neoplasms of various organs excluding the brain and the spinal cord. The age of the control subjects was 31-65 years with an average of 55. They were 5 men and 6 women. The elapsed time between death and autopsy was 2-6 h with the average of 3 h.

Precentral gray and white matters (Brodmann's area 4) weighing about 2 g were obtained and kept in $-80\,^{\circ}C$ until analyzed. Histopathology of the ALS motor cortex revealed a remarkable reduction in the number of Betz cells as well as other pyramidal cells, but there was neither marked gliosis nor demyelination. These changes in the motor cortex were obviously milder than those observed in the spinal cord[4].

Tissue was homogenized in ice-cold 1% picric acid and after centrifugation the supernatant was passed through a column of Dowex 2-X8 resin to remove picric acid. After freeze-drying the eluate, the final volume was adjusted by using a lithium citrate buffer of pH 2.15 to contain 30-200 mg of wet wt of tissue per ml. 1 ml of the final sample was applied to a JLC-6AH amino acid analyzer. Lithium citrate buffers were used in the analysis of neutral and acidic amino acids for the separation of glutamine and asparagine[6]. The value of each amino acid was expressed as μmoles per g wet wt of tissue.

Results. Among 28 free amino acids and related compounds quantitated in the ALS motor cortex, statistically significant changes were found in only 7 amino acids. Namely in the gray matter of the ALS precentral gyrus the contents of taurine, valine, leucine, isoleucine, phosphoserine and asparagine were increased. In the white matter of the same area, the contents of taurine, leucine, isoleucine and ornithine were increased. Among the amino acids changed, taurine was the only one which was increased without exception in the motor cortex of all the 3 ALS cases and the degree of its increase was most remarkable (tables 1 and 2).

Discussion. Histopathological changes in the motor cortex of the ALS patients were milder, as compared with those in the spinal cord. In a sense, the stage of the illness in the ALS motor cortex could be considered to be earlier than that in the spinal cord, where loss of nerve cells and gliosis were advanced. Actually only 7 amino acids were changed in the ALS motor cortex, while 16 amino acids were changed in the spinal cord[4]. Among these, taurine was the only amino acid which showed a common change in both the motor cortex of all the 3 ALS cases (tables 1 and 2) and the spinal cord[4]. In the spinal cord, taurine content was elevated rather diffusely with no restriction to the anterior column, anterior and lateral funiculi where histopathological changes were severe[4]. The same tendency was observed in the motor cortex where the increase in taurine content was common to both gray and white matters (tables 1 and 2). The branched-chain amino acids were increased in the ALS motor cortex (tables 1 and 2), but not at all in the ALS spinal cord[4], suggesting that these changes would not

Table 1. Free amino acids and related compounds in gray matter of the motor cortex from 3 patients with amyotrophic lateral sclerosis (ALS)

Precentral gray matter	Controls (11)	ALS patients (3)			
	Mean ± SD	Case 1	Case2	Case3	Mean ± SD
Glutamic acid	6.61 ± 2.82	5.86	3.45	5.24	4.85 ± 1.02
Glutamine	4.22 ± 1.89	3.32	6.11	4.37	4.60 ± 1.15
GABA	2.52 ± 0.61	2.47	2.16	3.52	2.72 ± 0.58
Aspartic acid	1.57 ± 0.78	1.39	0.72	1.84	1.32 ± 0.46
Alanine	1.07 ± 0.23	0.32[b]	1.45	1.75[a]	1.17 ± 0.62
Glycine	0.92 ± 0.32	1.28	0.99	0.94	1.07 ± 0.15
Taurine	0.86 ± 0.32	1.85[a]	1.73[a]	1.87[a]	1.82 ± 0.06[e]
Cystathionine	0.67 ± 0.34	0.68	0.62	0.50	0.60 ± 0.07
Serine	0.52 ± 0.17	0.61	0.50	0.50	0.54 ± 0.05
PEA	0.40 ± 0.12	0.21	0.52	0.49	0.41 ± 0.14
Threonine	0.33 ± 0.14	0.49	0.41	0.30	0.40 ± 0.08
Valine	0.26 ± 0.08	0.40	0.46[a]	0.39	0.42 ± 0.03[d]
Leucine	0.25 ± 0.09	0.51[a]	0.41	0.53[a]	0.48 ± 0.05[d]
Lysine	0.24 ± 0.08	0.30	0.53[a]	0.29	0.37 ± 0.11
Arginine	0.15 ± 0.05	0.31[a]	0.13	0.14	0.19 ± 0.08
Phenylalanine	0.14 ± 0.07	0.25	0.20	0.19	0.21 ± 0.03
Tyrosine	0.13 ± 0.07	0.21	0.16	0.16	0.18 ± 0.02
Histidine	0.12 ± 0.04	0.20	0.13	0.07	0.13 ± 0.05
Isoleucine	0.12 ± 0.05	0.26[a]	0.17	0.25[a]	0.23 ± 0.04[d]
Methionine	0.11 ± 0.04	0.19	0.06	0.05	0.10 ± 0.06
Proline	0.10 ± 0.04	0.14	0.14	0.12	0.13 ± 0.01
Phosphoserine	0.08 ± 0.02	0.11	0.12	0.14[a]	0.12 ± 0.01[c]
Asparagine	0.08 ± 0.02	0.12	0.11	0.16[a]	0.13 ± 0.02[d]
α-AnBA	0.06 ± 0.03	0.01	0.04	0.05	0.03 ± 0.02
Cystine/2	0.04 ± 0.02	0.01	0.03	0.01	0.02 ± 0.01
Ornithine	0.03 ± 0.01	0.03	0.06[a]	0.04	0.04 ± 0.01
β-Alanine	0.02 + 0.02	0.01	0.02	0.02	0.02 ± 0.00
Tryptophan	0.01 ± 0.01	0	0.01	0.01	0.01 ± 0.01
Total amount	21.64 ± 4.59				22.31 ± 1.02

Values expressed in μmoles per gram wet wt of tissue. GABA: γ-aminobutyric acid, PEA: phosphoethanolamine, α-AnBA: α-amino-normal-butyric acid. [a] Higher than 2 SD above the control mean. [b] Lower than 2 SD below the control mean. [c,d,e] Significantly higher than control at the levels of 5%, 1% and 0.1%, respectively, by Student's t-test.

Table 2. Free amino acids and related compounds in white matter of the motor cortex from 3 patients with amyotrophic lateral sclerosis (ALS)

Precentral white matter	Controls (11)	ALS patients (3)			
	Mean ± SD	Case 1	Case2	Case3	Mean ± SD
Glutamic acid	5.02 ± 2.03	4.26	2.30	3.56	3.37 ± 0.81
Glutamine	4.54 ± 1.54	4.98	6.80	4.25	5.34 ± 1.07
GABA	0.78 ± 0.26	0.74	0.58	1.03	0.78 ± 0.19
Aspartic acid	1.21 ± 0.43	1.40	0.80	0.98	1.06 ± 0.25
Alanine	0.94 ± 0.24	0.96	1.37	0.98	1.10 ± 0.19
Glycine	0.90 ± 0.32	1.04	1.03	0.69	0.92 ± 0.16
Taurine	0.94 ± 0.27	1.70[a]	2.07[a]	2.51[a]	2.09 ± 0.33[e]
Cystathionine	1.23 ± 0.47	1.06	0.85	0.76	0.89 ± 0.13
Serine	0.64 ± 0.19	0.76	0.58	0.44	0.59 ± 0.13
PEA	0.21 ± 0.06	0.16	0.24	0.32	0.24 ± 0.07
Threonine	0.34 ± 0.13	0.44	0.37	0.23	0.35 ± 0.09
Valine	0.20 ± 0.08	0.28	0.42[a]	0.17	0.29 ± 0.10
Leucine	0.20 ± 0.10	0.46[a]	0.43[a]	0.24	0.38 ± 0.10[c]
Lysine	0.23 ± 0.12	0.24	0.43	0.18	0.28 ± 0.11
Arginine	0.16 ± 0.08	0.32	0.11	0.08	0.17 ± 0.11
Phenylalanine	0.13 ± 0.07	0.18	0.16	0.12	0.15 ± 0.02
Tyrosine	0.12 ± 0.07	0.18	0.15	0.08	0.14 ± 0.04
Histidine	0.09 ± 0.03	0.14	0.11	0.04	0.10 ± 0.04
Isoleucine	0.09 ± 0.05	0.34[a]	0.24[a]	0.15	0.24 ± 0.08[d]
Methionine	0.10 ± 0.05	0.24[a]	0.04	0.05	0.11 ± 0.14
Proline	0.08 ± 0.03	0.10	0.10	0.08	0.09 ± 0.01
Phosphoserine	0.09 ± 0.02	0.06	0.14[a]	0.19[a]	0.13 ± 0.05
Asparagine	0.07 ± 0.02	0.04	0.07	0.08	0.06 ± 0.02
α-AnBA	0.05 ± 0.03	trace	0.03	0.01	0.01 ± 0.01
Cystine/2	0.03 ± 0.03	trace	0.02	0.01	0.01 ± 0.01
Ornithine	0.02 ± 0.01	0.12[a]	0.05[a]	0.04	0.07 ± 0.04[d]
β-Alanine	0.01 ± 0.01	0	0.02	0.01	0.01 ± 0.01
Tryptophan	0.01 ± 0.01	0	0.01	0	0.00 ± 0.00
Total amount	18.43 ± 3.64				18.97 ± 1.25

Values expressed in μmoles per gram wet wt of tissue. Same legends as in table 1.

continuously be observed in the disease process of ALS.

The mechanism of the accumulation of taurine in the ALS motor cortex, as well as in the spinal cord, is unknown. As to other amino acids, including the branched-chain amino acids, most of their increase might be a result of protein breakdown in the degenerative process. But this is unlikely in the case of taurine which is not a protein-composing amino acid. There is no evidence that glia cells have a greater taurine content than nerve cells. In addition, the elevation of taurine was also found in the precentral white matter (table 2) where no gliosis was observed. Postmortem changes[7], or other artifacts, are not conceivable in the increase in taurine content. Starvation[8] or protein-deficiency[9] seems not to induce the elevation of taurine in the brain, although there was a report indicative of it[10]. It is unlikely that our patient had been starved, since sufficient tube-feeding was performed, even at the end stages of the illness. The elevated level of taurine could be a reflection of some derangement of sulfur amino acid metabolism. As for other sulfur amino acids, methionine content was increased, but not constantly, in gray and/or white matters of the spinal cord[4] and of the motor cortex of the case 1 (table 2). The content of cystine was often increased in gray and white matters of the spinal cord, mainly in anterior areas[4], while it was not changed in the motor cortex. Cystathionine content was not changed at all.

So far there have been no reports indicating changes in sulfur amino acids in the ALS nervous tissue. However, the constant increase in taurine content, as well as occasional increase in the contents of methionine and cystine[4], suggest that the metabolism of sulfur amino acids in the nervous tissue might be affected in comparatively early stages of the disease process of ALS. Whether the elevated level of taurine is merely a secondary phenomenon, or has some bearing on the pathogenesis of ALS, remains a subject for further study.

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