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Amino acids acting as transmitters in amyotrophic lateral sclerosis (ALS)

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Objectives – In amyotrophic lateral sclerosis (ALS), a neurodegenerative disease of unknown origin, excitotoxic mechanisms are supposed to be involved. Divergent results are, however, presented either because of the heterogeneity of this disease, and/or different methodologies used to evaluate the excitotoxic amino acids content. The results of the most sensitive high performance liquid chromatography (HPLC) techniques with precolumn derivatization of fasting serum and CSF glutamate, aspartate, glycine and γ-aminobutyric acid (GABA) in mild and severely progressing ALS cases are presented here. *Material and methods* – We studied 25 ALS patients with different course of the disease and controls, which consisted of 10 cases with other motor neuron diseases and 20 healthy, age-matched subjects. *Results* – In the ALS patients with a mild course of the disease serum glutamate and aspartate content was either normal or slightly decreased, in all of these cases a rise in GABA and glycine was present. In the severely progressing ALS cases serum glutamate and aspartate was increased. The GABA content was either normal or increased, the glycine level appeared to be either normal or decreased. In CSF the amino acids changes in ALS were less pronounced as compared to serum. The most frequent finding was the increase in GABA concentration both in the mild and the severely progressing group. CSF glutamate in ALS patients with mild course of the disease was decreased, in the severely progressing cases the glutamate level appeared in a broad range from decreased to increased values. CSF aspartate was either normal or elevated, glycine values were present in a broad range from decreased to increased values. In the other tested motor neuron diseases no consistent changes in serum and CSF amino acids concentration was observed. *Conclusions* – The data from serum and CSF indicate that in ALS an imbalance between excitatory and inhibitory amino acids might be present in the brain, which may be induced in different ways in particular ALS patients. It may be an important factor for the mediation of neurons death.

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Selective degeneration of neurons in cerebral cortex, brainstem and spinal cord is the pathological hallmark in amyotrophic lateral sclerosis (ALS). The cause of these changes is still a mystery. One of the hypotheses holds that glutamate, the primary excitatory neurotransmitter in the central nervous system, accumulates at synapses to toxic concentrations and causes neurons to die, probably through a calcium-dependent pathway (1, 2). This hypothesis is supported mainly by abnormalities in glutamate metabolism (3, 4), a decreased high-affinity glutamate uptake by synaptosomes from the spinal cord and motor cortex (5) and decreased expression of the primarily glial GLT-1 glutamate transporter (6). Despite that this hypothesis is not commonly accepted there are therapeutic trials provided of

this disease with drugs which are able to modulate the glutamatergic system (7–10). The results of these trials are divergent. The usually presented opinion is, however, that using Riluzole, which is an anti-glutamate drug, it is possible to improve the survival of the ALS patients (11, 12).

We present here data on the content in serum and CSF of glutamate and aspartate, and also of GABA and glycine in ALS patients of mild and severe progression of this disease. The rationale behind the evaluation of the latter two amino acids is that they are involved in modulating of the excitotoxic effect of glutamate, and can cause an imbalance between excitation and inhibition in the brain. This in turn can mediate the cell death.

Material and methods

Patients

Twenty-five patients with ALS, 20 men and 5 women, were examined. The age of these patients was in the range between 26 and 66 years at diagnosis (the mean age was 51.0±11.9), all were sporadic without a family history of ALS. The diagnosis was based on WFN criteria (13), which allowed us to include patients with definite (n=18), probable (n=5), and suspected (n=2) ALS. At the time of examination all the ALS patients were ambulatory. The mean duration time of the disease ranged from 3 months to 12 years. Disability was scored using the Norris score (0 to 120). The course of the disease was mild in 10 patients (age 48.2±13.4, duration of the disease from 6 months to 12 years), 15 cases (age 52.9±10.8, duration of the disease from 3 months to 3 years) developed severe or moderate impairment in swallow, speech, breath, walking or upper limbs motor function within 2 years, according to the Norris score. The results in ALS were compared with disease controls, which consisted of 4 cases of multifocal motor neuropathy (MMN), 3 cases of Guillain–Barré syndrome (GBS) and 3 patients with sensorimotor neuropathy cases (SMN). The normal controls (n=20) were volunteers, aged 27 to 65 years, without generalized diseases.

Biochemical analysis

Blood and CSF specimens, obtained after an overnight fast, were immediately centrifuged at 3000 r.p.m. for 10 min. The samples were stored at –72°C until chromatography every next day. Before chromatography the samples were mixed with 4 vol of acidified methanol (8.4 ml 0.1 M HCl/100 ml methanol) and after 20 min at 4°C they were centrifuged for 10 min at 15,400×g and 4°C (14).

Amino acids analyses were performed by high performance liquid chromatography (HPLC) technique (15, 16). Reverse phase chromatography coupled with a switch to pre-column derivatization, employing the fluorescent ortho-phthaldialdehyde (17) reconstituted by β-mercapthoethanol (2 μl per ml of stock o-phthaldialdehyde) and a gradient system of 75 mM potassium phosphate buffer and methanol (pH 6.2), which progressively increases up to 100% of the proportion of organic modifier. A fluorescent detector with excitation at 330 nm and emission at 408 nm was used. The running time of each sample lasted 45 min. The HPLC system consisted of a Solvent Delivery System model 2800 (BioRad), Mobile Phase Conditioner M-3222 (BioRad), Fluorescence Detector Fluor 304 (Linear), Hyundai Delux Scan 15 PC Computer, ValueChrom™ Chromatography Software and a reverse-phase column Bio-Sil C18 HL 90–5 S (150×4.6 mm, particle size 5 μm, BioRad). Standard amino acids (Sigma), originally 17 mM in 0.1 M HCl, were run separately in concentrations between 25 to 550 pM per injection. The concentration of the amino acids was calculated from their fluorescence curves. Each standard was run in duplicate for each set of analysis.

Statistical analysis

The variances, comparison of means and significance analysis were performed by the Wilcoxon's test.

Results

A representative chromatogram of standard amino acids mixture is presented in Fig. 1. The concentration of glutamate and aspartate in serum of the mild progressing ALS cases was either normal or

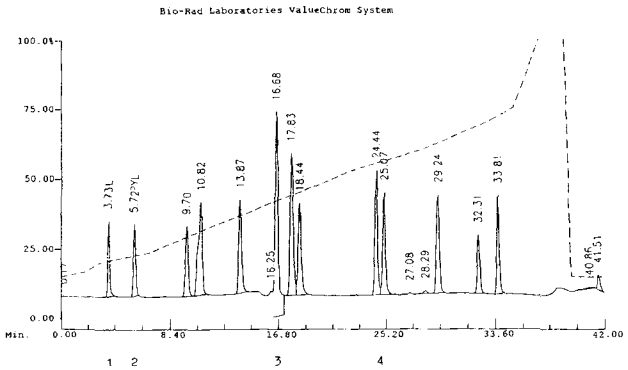

Fig. 1. Representative chromatogram of standard amino acids mixture: 1 – aspartate, 2 – glutamate, 3 – glycine, 4 – gamma-aminobutyric acid.