Abnormal Excitatory Amino Acid Metabolism in Amyotrophic Lateral Sclerosis

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Recently, the excitatory amino acid neurotransmitter glutamate was implicated in the pathogenesis of a variety of chronic degenerative neurological diseases in humans and animals. This report describes abnormalities in excitatory amino acids in the central nervous system of 18 patients with amyotrophic lateral sclerosis (ALS). The concentration of the excitatory amino acids glutamate and aspartate in the cerebrospinal fluid were increased significantly ($p < 0.01$) by 100 to 200% in patients with ALS. Similarly, the concentrations of the excitatory neuropeptide N-acetyl-aspartyl glutamate and its metabolite, N-acetyl-aspartate, were elevated twofold to threefold in the cerebrospinal fluid from the patients. There was no relationship between amino acid concentrations and duration of disease, clinical impairment, or patient age. In the ventral horns of the cervical region of the spinal cord, the level of N-acetyl-aspartyl glutamate and N-acetyl-aspartate was decreased by 60% ($p < 0.05$) and 40% ($p < 0.05$), respectively, in 8 patients with ALS. Choline acetyltransferase activity was also diminished by 35% in the ventral horn consistent with motor neuron loss. We conclude that excitatory amino acid metabolism is altered in patients with ALS. Based on neurodegenerative disease models, these changes may play a role in motor neuron loss in ALS.

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Amyotrophic lateral sclerosis (ALS) is a chronic progressive disease of selective upper and lower motor neuron degeneration, whose pathogenesis is unknown. In several other chronic neurological disorders, such as olivopontocerebellar atrophy [1, 2] and Huntington's disease [3, 4], abnormalities of glutamate metabolism have been shown to occur and are thought to play a role in the pathophysiology of the disorder. Glutamate, the primary excitatory neurotransmitter in brain, can exert specific neurotoxic effects and can induce neuronal degeneration in vivo and in vitro [5–8]. Of particular interest, excitatory neurotoxins derived from the ingestion of cycad nuts may be, in part, responsible for the motor neuron degeneration associated with the ALS-parkinsonism-dementia complex of the Chamorro population of the Mariana islands [9, 10]. Based on the models of environmental excitatory neurotoxins [9, 10] and prior studies of human brain and spinal cord [11–16], we hypothesized that the metabolism of excitatory amino acids might be altered in patients with ALS.

To test this possibility, we examined the concentrations of glutamate, aspartate, and the excitatory neuropeptide N-acetyl-aspartyl glutamate (NAAG) in the cerebrospinal fluid (CSF) and spinal cords of patients with well-characterized, classic ALS. We report significant and specific increases in the concentrations of these excitatory compounds in the central nervous system (CNS) of patients with ALS.

Materials and Methods

The study was divided into two parts: (1) analysis of CSF from living patients and (2) analysis of autopsy material from the cervical region of the spinal cord. All biochemical analyses were performed in a blinded fashion.

CSF Analysis

CSF was collected and compared in three groups of patients (Table 1): 18 patients with ALS, 18 patients with other neurological diseases (OND), and 10 patients with hepatic encephalopathy (a comparison group expected to have high CSF levels of glutamine, to control for artifactual elevation of CSF glutamate levels).

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Table 1. Patient Characteristics in CSF Studies

Group	No.	Age (mean yr ± SEM)[a]	Duration of Disease (mo)[a]	Diagnoses[b]
Amyotrophic lateral sclerosis	18	52 ± 3.6 (31–75 yr)	15 ± 2.6 (6–36)	—
Control	18	53 ± 3.8 (22–80)	—	1° Biliary cirrhosis (2) Brainstem cerebrovascular accident (1) Cerebellar ataxia (1) Cervical myelopathy (2) Chronic inflammatory demyelinating polyneuropathy (1) Hepatolenticular degeneration (1) Low back pain (1) Lumbar stenosis (1) Migraine (3) Multiple myeloma (1) Neurosyphilis (1) Pseudotumor cerebri (2) Progressive supranuclear palsy (1)
Hepatic encephalopathy	10	47 ± 4.9 (33–65)	—	—

[a] Values in parentheses represent ranges.
[b] Values in parentheses represent number of patients.

With informed consent lumbar punctures were performed in the lateral decubitus position at the L3 to L4 or L4 to L5 interspace. CSF was collected for the following studies: determinations of glucose and protein levels, cell count, and amino acid analysis. In addition, an aliquot of the first 10 ml of CSF was placed on ice immediately after lumbar puncture and either assayed for amino acids or stored at −80°C. A protocol for these studies was previously approved by The Johns Hopkins Institutional Review Board.

PATIENTS WITH ALS. We collected CSF from those patients with ALS who had lumbar puncture performed in the 2-year period from 1986 to 1988. The diagnosis of ALS was based on a rigorous set of criteria designed for a therapeutic trial now under way in our institution. Evaluations included a detailed history and physical examination, and extensive hematological, biochemical, electrophysiological, and radiological testing. The diagnosis required the presence of both upper and lower motor neuron signs, clear evidence of progression, normal nerve conduction velocities and late responses, and electromyographic evidence of diffuse denervation [17, 18]. Patients diagnosed with ALS also met an extensive list of exclusionary criteria including sensory findings; unexplained bowel or bladder changes; and anatomical, metabolic, or toxic disorders that could mimic ALS, e.g., myelopathy, lead intoxication, endocrine abnormalities, hexosaminidase A deficiency, or peripheral neuropathy. After examination of the patient's record by four neurologists, the diagnosis was accepted or rejected by consensus.

DISEASE CONTROL SUBJECTS. CSF from control patients with OND was obtained at the time of diagnostic lumbar puncture. Control patients with adequate available CSF were selected to include some patients with diseases marked by neuronal death or degeneration (see Table 1).

HEPATIC ENCEPHALOPATHY. For amino acid analysis of CSF, we studied an important comparison group with hepatic encephalopathy. Since CSF levels of glutamine are greatly elevated in that disorder [19], it provides an internal control in the study to resolve concern about artifactual elevated concentrations of CSF glutamate arising by breakdown from glutamine. All patients had chronic recurrent encephalopathy graded as clinical stage 1 or 2 [20]. All had cirrhosis, proved by evaluation of biopsy specimens, due to chronic hepatic diseases that included chronic active hepatitis, Laennec's cirrhosis, primary biliary cirrhosis, or Wilson's disease. Evidence for chronic hepatocellular disease was based on the following: presence of ascites, history of esophageal varices, decreased serum concentration of albumin (< 3.0 mg/100 ml), prothrombin ratio greater than 1.2, serum bilirubin concentration of greater than 2.5 mg/100 ml. Hepatic encephalopathy was precipitated in most patients by either gastrointestinal bleeding or increased intake of dietary protein.

Analysis of Spinal Cord Sections

SUBJECTS. Sections from the cervical region of the spinal cord were obtained at autopsy from two study groups: The ALS group consisted of 8 patients with ALS diagnosed as just described. A control group was composed of 8 patients with nonneurological disease and 1 patient with Parkinson's disease (Table 2). Both the ALS and control spinal cord specimens came from The Johns Hopkins Brain Bank (except specimens from 2 control cases were kindly supplied by the National Neurological Research Bank, Los Angeles, CA). All tissue was stored up to 2 years at −80°C. Mean age and postmortem delay were similar for both groups.

POSTMORTEM TISSUE. Analysis of the specimens was performed on 1-mm micropunch samples from ventral gray matter.

HISTOLOGY. Spinal cord specimens were routinely fixed in formalin, and then sectioned and stained with hematoxylin and eosin for pathological verification of ALS. Control spinal tissue was similarly examined.

Table 2. Patient Characteristics in Spinal Cord Studies

Group	No.	Age (mean yr ± SEM)[a]	Postmortem Delay (mean hr ± SEM)[a]	Duration of Disease (mo)[a]	Diagnoses[b]
Amyotrophic lateral sclerosis	8	62 ± 7.2 (51.0–71.0)	11 ± 6.4 (3.5–18.0)	27 ± 15 (12–48)	—
Control	9	61 ± 26 (0.5–87.0)	10.6 ± 5.5 (6.0–21.5)	—	Myocardial infarction (5) Leukemia (1) Sepsis (1) Multiple myeloma (1) Parkinson's disease (1)

[a]Values in parentheses represent ranges.
[b]Values in parentheses represent number of patients.

Amino Acid and Enzyme Assays

Aliquots of spinal cord homogenates were assayed for total protein by the method of Lowry and associates [21].

AMINO ACID ANALYSIS. Amino acid analysis of sulfosalicylic acid–treated CSF samples was performed by automated ion-exchange chromatography with lithium-based buffers on a Beckman 6300 amino acid analyzer (Beckman Instruments, Fullerton, CA). Several aliquots were frozen and assayed at several times after the original assay, to evaluate stability of the amino acids. Evaluation of ALS and control CSF samples was done in parallel in the same assay.

N-ACETYL-ASPARTYL GLUTAMATE AND N-ACETYL-ASPARTATE ANALYSIS. NAAG and N-acetyl-aspartate (NAA) were measured in CSF and spinal cords of patients with ALS and control patients by high-performance liquid chromatography (HPLC) and in the case of NAAG, confirmed by radioimmunoassay utilizing antiserum to NAAG [22, 23]. Due to limited CSF availability, NAAG and NAA were quantified in only 12 of the 18 patients with ALS.

N-ACETYLATED-α–LINKED ACIDIC DIPEPTIDASE MEASUREMENTS. Activity of the NAAG metabolizing enzyme N-acetylated-α-linked acidic dipeptidase (NAALADase) was evaluated by measuring the hydrolysis of N-acetyl-L-aspartyl-L-[^{3}H]glutamate as described by Robinson and colleagues [22].

CHOLINE ACETYLTRANSFERASE ACTIVITY. Choline acetyltransferase (ChAT) activity in the spinal cord was determined in tissue homogenates as described by Blakely and colleagues [23]. In brief, the assay measures the synthesis of ^{14}C-acetylcholine after separation from ^{14}C-acetyl-coenzyme A (CoA) by anion exchange (AG1-X8, chloride$^-$ form) liquid chromatography.

GLUTAMATE DECARBOXYLASE ACTIVITY. Glutamate decarboxylase activity was measured in lateral cervical hemisections of the spinal cord by the method of McDonnell and Greengard [24]. The assay quantified $^{14}CO_2$ released from the α-carboxy–labeled ^{14}C-glutamate.

Statistical Analysis

Statistical analysis of data was performed by analysis of variance and *t* tests, with correction for unequal variances when appropriate, using the SAS General Linear Models Procedure (SAS Institute, Cary, NC). Values are expressed as mean ± standard error of the mean (SEM).

Table 3. CSF Amino Acid Analysis[a]

Amino Acid	Amyotrophic Lateral Sclerosis	Control	% Change
Aspartic acid	8.4 ± 1.2[b]	4.2 ± 0.6	100
Threonine	44.4 ± 3.5[c]	32.8 ± 2.0	35
Serine	55.8 ± 6.1[c]	41.1 ± 2.2	36
Asparagine	5.7 ± 0.8	5.5 ± 0.2	4
Glutamic acid	8.4 ± 1.4[b]	2.9 ± 0.4	190
Glutamine	512.6 ± 44.7	551.0 ± 19.0	−7
Glycine	26.6 ± 3.7	19.3 ± 2.7	38
Alanine	50.2 ± 4.9	38.8 ± 2.8	29
Valine	21.7 ± 1.5	18.2 ± 1.3	19
Cystine	1.7 ± 0.1	1.8 ± 0.1	6
Methionine	2.7 ± 0.4	2.7 ± 0.2	0
Isoleucine	6.9 ± 0.6	6.1 ± 0.4	13
Leucine	14.9 ± 0.9	13.7 ± 0.9	9
Tyrosine	12.5 ± 1.0	11.6 ± 1.0	8
Phenylalanine	11.6 ± 1.8	10.7 ± 0.8	8
Lysine	33.2 ± 2.7[c]	25.9 ± 1.5	28
Histidine	18.1 ± 2.0	17.8 ± 1.6	2
Arginine	25.7 ± 3.0	19.9 ± 1.0	29

[a]Values are μmol/liter (mean ± SEM).
[b]$p < 0.01$ versus control values.
[c]$p < 0.05$ versus control values.

Results

Histopathological analysis confirmed the diagnosis of ALS in all autopsy specimens. There was a characteristic loss of large motor neurons in the ventral horns of the gray matter in the cervical sections. All control specimens demonstrated intact, normal-appearing, large motor neurons in the ventral gray matter.

CSF Amino Acids, NAAG, and NAA

CSF amino acid levels in the control subjects in this experiment were not significantly different from previously published normal levels (Table 3) [25, 26]. There were no significant differences in either CSF glucose or protein levels or cell counts between any of the groups examined.

In patients with ALS, the most striking change was

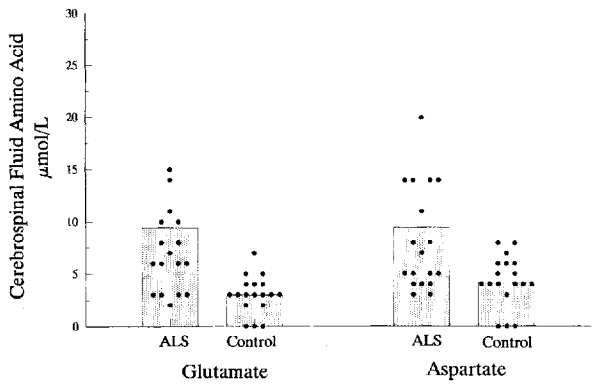

Fig 1. Concentrations of glutamate and aspartate in CSF from 18 patients with amyotrophic lateral sclerosis (ALS) compared with those found in 18 control subjects. Shaded bars represent group mean.

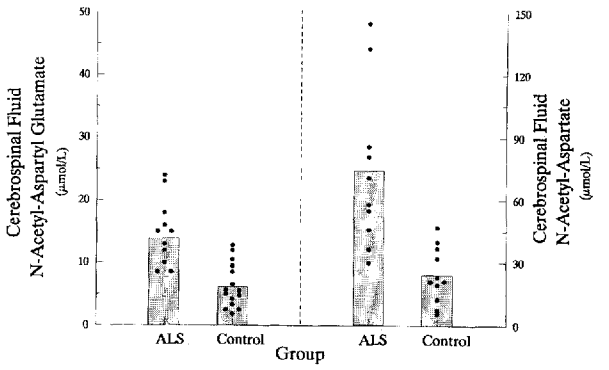

Fig 2. N-Acetyl-aspartyl glutamate and N-acetyl-aspartate concentrations in CSF from control patients and patients with amyotrophic lateral sclerosis (ALS). Shaded bars represent group mean.

the marked increase in the concentrations of the excitatory amino acids glutamate and aspartate. The average CSF concentration of glutamate was increased almost threefold ($p < 0.01$) in patients with ALS and up to tenfold in individual patients (Fig 1). Aspartate concentration was doubled ($p < 0.01$) in CSF from patients with ALS, with individual levels elevated as much as fivefold.

Concentrations of NAAG and NAA were both significantly elevated in the CSF of patients with ALS (Fig 2). NAAG concentration was more than doubled in CSF from patients with ALS ($p < 0.01$) compared to control specimens, with concentrations in individual samples increased up to fourfold. Similarly, NAA concentration was increased threefold in the patients with ALS ($p < 0.01$), again with individual levels elevated greater than fivefold compared to levels in the control CSF. NAALADase activity could not be detected in CSF specimens from either patients with ALS or control subjects.

There was no significant correlation between duration of disease and CSF aspartate, glutamate, NAAG, or NAA concentration. Furthermore, there was no observable relationship between these compounds and overall disability score or predominant pattern of dysfunction (upper or lower motor neuron signs) at the time of CSF collection.

By comparison, in 10 patients with Huntington's disease, CSF concentrations of NAAG and NAA were not different from control levels (Kurlan, Tsai, Shoulson, and Coyle, unpublished data).

There were small (< 40% above control values), statistically significant ($p < 0.05$) increases in the concentrations of the hydroxyamino acids serine and threonine in CSF from patients with ALS (see Table 3). In CSF from patients with ALS there were no significant changes in the levels of aliphatic or branched chain amino acids (alanine, glycine, valine, isoleucine, and leucine), in aromatic amino acids (tyrosine and phenylalanine), or in glutamine. Levels of basic amino acids (lysine, histidine, and arginine) were unchanged in ALS specimens, with the exception of lysine which was slightly increased by 28% ($p < 0.05$).

Amino acid concentrations are not shown for citrulline, phosphoethanolamine, hydroxyproline, α-amino-N-butyric acid, cystathione, ornithine, 1-methyl-histidine, and 3-methyl-histidine; however, they were not different between control and ALS samples of CSF.

Control Studies of Amino Acids in CSF

Glutamine is hydrolyzed to glutamate, and spontaneous hydrolysis could theoretically increase the levels of glutamate artifactually. To control for this possibility, samples of CSF from patients with hepatic encephalopathy, with marked elevations in glutamine concentrations, were also analyzed. Analysis of CSF amino acids revealed that, similar to previously published reports for chronic liver disease, glutamine was increased 2.3-fold (1,285 ± 210 μmol/liter) in patients with hepatic encephalopathy as compared to control CSF samples [19, 27]. In samples processed identically to ALS samples, the glutamate and aspartate concentrations remained normal in the CSF from patients with hepatic encephalopathy, in spite of marked elevations of glutamine. In accordance with other CSF studies, a number of CSF amino acids in patients with hepatic encephalopathy were increased; these included tyrosine, phenylalanine, methionine, threonine, and histidine (data not shown) [27].

Samples of CSF subjected to repeated analyses over a 9-month period revealed no systematic change in CSF glutamate or aspartate concentrations.

Spinal Cord Analysis

The average level of NAAG in the ventral gray horns of patients with ALS was significantly decreased by

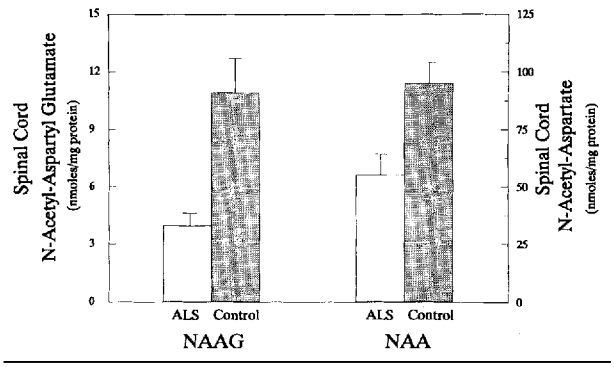

Fig 3. Concentrations of N-acetyl-aspartyl glutamate (NAAG) and N-acetyl-aspartate (NAA) in the ventral horn of patients with amyotrophic lateral sclerosis (ALS) and in control subjects. Data are presented as mean ± SEM.

Table 4. Spinal Tissue Analysis[a]

	Amyotrophic Lateral Sclerosis (n = 8)	Control (n = 9)
Choline acetyltransferase	971 ± 98[b]	1,522 ± 194
Glutamate amino decarboxylase	695 ± 92	513 ± 164
N-acetylated-α–linked acidic dipeptidase	0.148 ± 0.021	0.167 ± 0.035

[a] Values are pmol/mg of protein/hr (mean ± SEM).
[b] $p < 0.05$ compared to control values.

60% ($p < 0.02$), compared to levels in the same anatomical regions in the control cords (Fig 3). Likewise, NAA levels in ventral horn tissue of patients with ALS were decreased by 40% ($p < 0.05$). Control levels of NAAG and NAA were similar to those previously published [15]. There was no statistical relationship between NAAG levels and either disease duration or patient age.

Spinal cord NAALADase activity, present primarily in synaptic membranes, was similar in both ALS and control tissue (Table 4). Similarly, the activity of the enzyme glutamate decarboxylase, a general marker for intrinsic spinal gamma-aminobutyric acid (GABA) neurons, was unchanged in ALS spinal tissue compared to control tissue (see Table 4). By contrast, activity of the enzyme ChAT, which is present primarily in the large spinal α-motor neurons, was decreased 36% in ventral gray matter tissue compared to similar regions in control spinal cord, as expected. There was no significant correlation between the loss of ChAT activity and the spinal cord concentrations of NAAG or NAA.

Discussion

Our study showed that concentrations of the excitatory amino acids aspartate and glutamate and the neuropeptide NAAG are selectively elevated twofold to threefold in the CSF of patients with ALS. This is consistent with findings from a number of other studies that suggested alterations in the metabolism of the excitatory amino acids aspartate and glutamate in ALS [11, 12]. In postmortem studies, tissue levels of aspartate, glutamate, and GABA were reported to be diminished in several brain and spinal cord regions in ALS [12, 14, 16, 28]. In addition, studies of leukocytes and plasma have suggested that glutamate metabolism is altered in patients with ALS [29, 30]. However, study of CSF affords the advantages of allowing in vivo measurements of these amino acids within the CNS and permits studies in the early development of disease rather than at the end stage, as in postmortem analyses. Two surveys of CSF amino acids performed 12 and 20 years ago on patients with a wide variety of metabolic and degenerative diseases demonstrated somewhat elevated glutamate levels in the CSF of a small number of patients with motor neuron disease [26, 31]. Our study confirms this early finding in ALS, and extends it to the excitatory amino acid aspartate and to NAAG.

NAAG is a neuropeptide found in high concentrations in the CNS, in an uneven distribution with highest levels in the spinal cord and caudal region of the brainstem [32–34]. Immunohistochemical analysis demonstrated colocalization of NAAG to glutamatergic neurons in motor cortex, olfactory mitral cells, primary sensory afferent neurons, and lateral vestibular nuclei [35–37]. In addition, it also localizes to ChAT-positive motor neurons of both spinal and cranial nuclei, as well as some noradrenergic and adrenergic nuclei in the pons and medulla, and some serotonergic raphe nuclei [32, 38]. NAAG has been shown to be released from neural tissue by calcium-dependent processes or by electrical depolarization [39–41]. It exhibits relatively weak postsynaptic effects [42]. Following synaptic release, NAAG is metabolized via NAALADase to glutamate and NAA [22, 34]. Thus, NAAG has the properties of a neuromodulator and may be an additional source of the neurotransmitter glutamate [34].

Increased CSF levels of NAAG, NAA, and glutamate suggest the possibility of increased release and metabolism of these compounds in patients with ALS. One interpretation would be that increased release of NAAG occurs in ALS, with subsequent metabolism to glutamate and NAA leading to increased CSF concentrations of these compounds. Since the activity of NAALADase was normal in spinal cord sections from patients with ALS, the possibility that this was due to accelerated NAAG catabolism is less attractive. The low levels of NAAG in the ventral horn of the cord,

consistent with a finding described in an abstract [15], may be due to at least two factors: increased release of NAAG, perhaps by descending motor tracts, with resultant tissue depletion; or loss of α-motor neurons containing NAAG; or both. Whether these changes represent primary abnormalities in glutamate or NAAG metabolism or are a consequence of motor neuron death is not yet certain. In that regard, ChAT levels were decreased in the ventral horn of the spinal cord, as demonstrated by others [43, 44], which reflects the loss of large cholinergic motor neurons as seen on histopathological examination. Though levels of both NAAG and ChAT were reduced in the ventral horn, their lack of intercorrelation does not support the idea that the alteration in NAAG metabolism in CNS is merely secondary to motor neuron loss. The normal levels of GAD reflect intact GABA neurons in ALS spinal tissue.

The increased concentration of excitatory amino acids that we found in CSF from patients with ALS may be physiologically meaningful. In acute cerebral ischemia, extracellular glutamate concentrations increase to levels comparable to those we observed in the CSF from our patients with ALS [45]. Furthermore, the increased CSF concentration of glutamate to the 10 μmol range could be neurotoxic. There have been a large number of studies demonstrating acute glutamate toxicity in cultured neurons. Utilizing primary cerebellar granule cells, Favaron and colleagues observed 50% cell death with glutamate concentrations as low as 10 μmol.

Abnormal CSF concentrations of glutamate and aspartate may suggest altered neurotransmitter metabolism; however, only studies that are specifically directed at the neurotransmitter compartment can address these questions. The relationship between changes of CSF levels of glutamate, aspartate, and NAAG and excitatory neurotransmitter function is complex for two main reasons: (1) Glutamate metabolism in the CNS occurs in a two-compartment system and (2) CSF concentration of a compound reflects multiple processes, including altered release, uptake, cell death, or altered CSF resorption kinetics [47, 48]. Experimental studies of brain glutamate suggest that metabolism occurs in two metabolic compartments: a large compartment that is primarily for general cellular metabolism and a small compartment (10% of total) that is for neurotransmitter metabolism and is localized to the presynaptic endings and astrocytes [49–51]. Thus, measurement of total glutamate or aspartate concentration in brain tissue does not necessarily reflect the metabolism of the neurotransmitter component. At present we do not have the tools to evaluate the neurotransmitter compartment directly. Other approaches include assessing postsynaptic receptor function, and such studies are currently under way.

Previous studies demonstrated slight increases in several CSF amino acids in a number of chronic neurological diseases, e.g., Parkinson's disease [52–54]. However, the large changes in CSF concentrations of NAAG, glutamate, and aspartate reported here appear to be unique. These changes were not seen in our other patients with chronic degenerative diseases such as Huntington's disease and progressive supranuclear palsy, with acute cell damage during brainstem stroke, or with chronic spinal injury as in cervical myelopathy, and thus would suggest disease specificity to our observations.

The elevated CSF concentrations of serine, threonine, and lysine reached statistical significance in this and previous studies on motor neuron disease, but the degree of elevation was slight [11, 25, 31]. Although CSF concentrations of some amino acids may increase with age [52, 55], there was no significant difference in age between groups in our experiments.

If excitotoxicity contributes to the pathogenesis of motor neuron damage in ALS, then therapies designed to interfere with glutamate neurotransmission may be useful. To that end, Plaitakis and associates recently demonstrated that in a small group of patients with ALS, oral treatment with branched chain amino acids (valine, leucine, and isoleucine) was associated with slowing in the loss of strength [56]. The rationale for this treatment protocol was based on the potential neurotoxicity of the neurotransmitter glutamate and the assumption that administration of branched chain amino acids might alter neurotransmitter glutamate metabolism. Whether the administration of branched chain amino acids actually alters the metabolism of glutamate, aspartate, or NAAG remains to be investigated.

Excitatory amino acids and excitatory neuropeptides may be quite important in the pathogenesis of other degenerative neurological diseases. Canavan's disease, a chronic degenerative neurological disorder, was recently linked to deficiency in the enzyme that degrades NAA, aspartoacylase [57]. Two exogenous toxins that interact with glutamate receptors can also produce neuronal damage. Chronic exposure to β-N-methylamino-L-alanine, an amino acid derivative of *Cycas circinalis,* which was shown to be toxic to neurons in vitro [5, 8], appears also to cause a degenerative neurological syndrome that has some of the clinical and neuropathological characteristics of ALS [9, 10]. Furthermore, lathyrism, an upper motor neuron disorder seen after excessive ingestion of the chickling pea *(Lathyrus sativus),* may be caused by β-N-oxalylamino-L-alanine, a potent neurotoxic stereospecific glutamate analog [9].

By analogy, the pathophysiology of ALS may be based, in part, on the abnormal chronic exposure of neurons to excitotoxic substances, such as glutamate, glutamate analogs, or excitatory neuropeptides. Increased synaptic levels of glutamate acting directly, or

acting in the absence of other "protective" mechanisms, could be responsible for neuronal death in ALS. This study demonstrates that high levels of excitatory amino acids are indeed present in the CSF of patients with ALS. These substances may, in part, be responsible for the pathogenesis of motor neuron damage. The selective loss of motor neurons in the presence of these potential excitotoxins, however, remains a puzzling question.

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