Amino Acids (1996) 11: 37–42

Amino
Acids

Effects of branched-chain amino acids on plasma amino acids in amyotrophic lateral sclerosis

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Accepted August 17, 1995

Summary. Although the cause of amyotrophic lateral sclerosis (ALS) remains unknown, biological findings suggest that the excitatory amino acid glutamate contributes to the pathogenesis of ALS. In previous studies of ALS, the therapeutic effect of the branched-chain amino acids (BCAAs) leucine, valine and isoleucine has been evaluated. The present study aimed at investigating the acute effect of BCAAs on plasma glutamate levels in ALS patients. Following two oral doses of BCAAs, significantly increased plasma levels were seen for valine (500%), isoleucine (1,377%) and leucine (927%), however the plasma level of glutamate was not affected. The plasma level of several other amino acids (tryptophan, tyrosine, phenylalanine and methionine) were found decreased after oral BCAAs, which may indicate a diminution in the rate of degradation of muscle protein and/or an increase in tissue disposal of amino acids.

Keywords: Amyotrophic lateral sclerosis – Glutamate metabolism – Branched-chain amino acids

Introduction

ALS is a progressive neurodegenerative human disease of unknown pathogenesis, which is characterized by a selective degeneration of upper and lower motor neurons. Recently, reports (Plaitakis, 1987; Perry, 1990; Iwasaki, 1992) have shown abnormal glutamate metabolism in ALS and a role for this amino acid in the pathophysiological process of the disease has been postulated (Munsat, 1990).

Glutamate, the primary excitatory neurotransmitter in the brain, can exert specific neurotoxic effects through a well-described cascade of cationic and second-messenger events and can induce neuronal degeneration in vivo and in vitro (Choi, 1988). Glutamate is metabolized in part by the enzyme glutamate dehydrogenase that interconverts glutamate and α-ketoglutarate.

The activity of the enzyme has been reported as normal (Plaitakis, 1987) or reduced (Hugon, 1989) in ALS-leukocytes, and elevated in ALS dorsal horns of spinal cord (Malessa, 1988). Because glutamate dehydrogenase can be activated in vitro by the branched-chain amino acids (BCAAs) L-leucine and L-isoleucine (Yielding, 1961), BCAAs have been used for the treatment of ALS. In a pilot study (Plaitakis, 1988) BCAAs significantly benefitted ALS patients in terms of maintenance of extremity muscle strength and continued ability to walk, but in two other studies (Testa, 1989; Beghi, 1993) no statistically significant differences were found in the clinical outcome between the patients treated with BCAAs and the control groups.

Recently it has been shown (Gredal, 1995) that subchronic BCAAs treatment did not significantly affect plasma glutamate levels in ALS patients at basal conditions or during glutamate loading (60 mg/kg). To further evaluate the effect of BCAAs on amino acids metabolism in ALS patients, we decided to study the acute effect of two doses of BCAAs on the plasma levels of glutamate and other amino acids.

Material and methods

ALS-patients

Six ALS patients (5 males and 1 female) with mean age 57 years, range 44–66 years, were studied. The study was approved by the Scientific-Ethical Committee for the Municipalities of Copenhagen and Frederiksberg.

The diagnosis was established by clinical criteria (signs of both upper and lower motor neuron symptoms and history of progression for at least 3 months) and electromyographical criteria (evidence of acute and chronic denervation involving limb and axial musculature). The mean disease duration was 15 months, range 8–20 months. All patients had symptoms from the arms and legs when they entered the study and only one patient showed bulbar involvement.

Design

After an overnight fast, the patients received at two days intervals either water alone or two oral doses with one hour interval of a mixture of 3 g L-leucine, 2 g L-isoleucine, and 1.6 g L-valine (Val), as prescribed by Plaitakis (1988), in 200 ml water.

Amino acids analysis

Amino acids were analyzed according to Møller (1993). Venous blood samples were collected in EDTA tubes before and every 15 min until 3 h after BCAAs consumption. After centrifugation at 2,000 × g for 15 min at 4°, the plasma samples were stored at −80° until analysis. Samples were protein precipitated by adding a cooled solution of sulfosalicylic acid. Amino acids were assayed by ion exchange chromatography using *o*-phthaldialdehyde postcolumn derivatization and fluorometric detection.

Statistical analysis

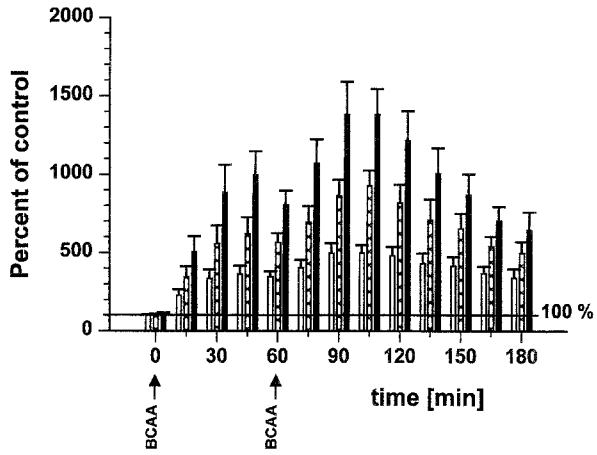
Basal levels of amino acids were evaluated by two-tailed Student's t-test for paired data, whereas the plasma levels during the three hours after BCAAs consumption were evaluated by analysis of variance (ANOVA) and further more by Duncan's multiple-range test. P values above 0.05 were regarded as not significant (ns).

Results

In the two tests, water and BCAAs, ALS patients showed comparable basal plasma amino acids levels e.g. glutamate 42 ± 12 and 45 ± 11 μmol/l (mean ± SD).

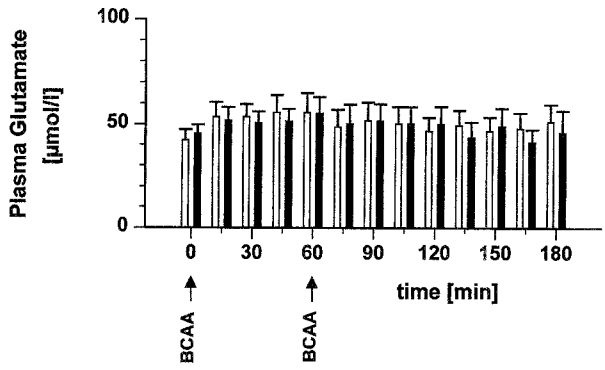
Following the two doses of BCAAs a significant treatment effect ($p << 0.001$) was found with increased plasma Val, Iso and Leu levels. Figure 1 shows the increase in percent of control level for Val, Iso and Leu after two doses of BCAAs. Maximum concentrations (mean ± SD) of Val, Iso and Leu were 1,066 ± 249 μmol/l (500%), 787 ± 224 μmol/l (1,377%) and 1,093 ± 282 μmol/l (927%), respectively.

Figure 2 shows plasma glutamate levels (mean + SEM) versus time following water and two oral doses of BCAAs in ALS patients. No significant treatment effect was found for plasma glutamate, taurine, citrulline, and



Val; Anova: Treatment F [1,128] = 378; p << 0.001
Time F [12,128] = 4.9; p < 0.001
Leu; Anova: Treatment F [1,128] = 400; p << 0.001
Time F [12,128] = 5.5; p < 0.001
Iso; Anova: Treatment F [1,129] = 392; p << 0.001
Time F [12,128] = 6.0; p < 0.001

Fig. 1. Plasma levels of valine (open bars), leucine (hatched bars) and isoleucine (solid bars) (mean + SEM in percent of mean control) versus time in ALS patients following BCAAs consumption. All levels except basal levels differ significantly ($p < 0.01$) by the Duncan's multiple-range test



BCAAs compared to water;
Anova: Treatment F [1,129] = 0.2; p = 0.68
Time F [12,129] = 0.4; p = 0.95

Fig. 2. Plasma glutamate level (mean + SEM) versus time in ALS patients following water (open bars) and BCAAs (solid bars)

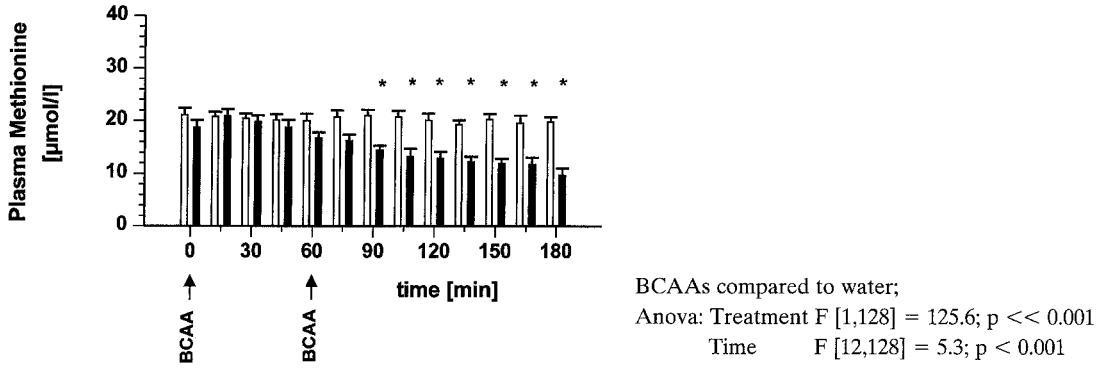


Fig. 3. Plasma methionine level (mean + SEM) versus time in ALS patients following water (open bars) and BCAAs (solid bars). * indicate $p < 0.01$ (Duncan's multiple-range test)

Table 1. Plasma amino acids in ALS patients (N = 6) after 2 doses of BCAAs

Amino Acid	ANOVA p-value	% of control at 180 min
Taurine	0.73	–
Glutamate	0.68	–
Citrulline	0.34	–
Alanine	0.10	–
Glutamine	0.03	102^{ns}
Threonine	0.011	81^{ns}
α-Amino-butyric acid	0.003	79^{ns}
Cysteine*	0.002	98^{ns}
Glycine	0.002	81^{ns}
Serine	<0.001	74^{ns}
Asparagine	<0.001	$78^{0.05}$
Tryptophan	<0.001	$66^{0.05}$
Tyrosine	<0.001	$61^{0.01}$
Phenylalanine	<0.001	$57^{0.01}$
Methionine	<0.001	$49^{0.01}$

*N = 3, Upper case: p-value by the Duncan's range test

alanine, whereas significantly decreased levels (up to fifty percent at 180 minutes) were seen for threonine, α-amino-butyric acid, cysteine, glycine, serine, asparagine, tryptophan, tyrosine, phenylalanine and methionine (Table 1 and Fig. 3). Only glutamine showed a significant main effect for treatment with increased plasma levels (Table 1).

Discussion

BCAAs supplementation has been used in treatment of latent portosystemic encephalopathy (Plauth, 1993) and in sepsis (Jimenez, 1991, 1992) to normalize amino acid metabolism. In sepsis (Jimenez, 1991, 1992) and during intense exercise (Blomstrand, 1992) there are found an increase in the plasma concentration of aromatic amino acids and a decrease in BCAAs, and it is suggested

that intake of BCAAs in such conditions can prevent or decrease the net rate of protein degradation in the muscles.

In ALS-patients only abnormality in the metabolism of glutamate has been described, but not in the aromatic or branched-chain amino acids metabolism. The treatment rationale for the use of BCAAs in ALS has been to activate glutamate dehydrogenase in order to normalize the glutamate metabolism.

Glutamate dehydrogenase is located in the mitochondria of several tissues e.g. brain, liver and kidney. Millimolar concentration of Leu and Iso are potent activators of glutamate dehydrogenase and glutamate metabolism in vitro (Yielding, 1961; Erecinska, 1990). In the present study activator concentrations in plasma of Leu was obtained after two doses of BCAAs, however no effect of BCAAs on plasma glutamate levels in ALS patients was found.

The BCAAs administration to ALS patients resulted in marked reductions in the plasma concentrations of the aromatic amino acids (phenylalanine, tyrosine, tryptophan) and methionine according with the findings of others in healthy subjects (Eriksson, 1981; Hagenfeldt, 1980), indicating a diminution in the rate of degradation of muscle protein and/or an increase in tissue disposal of amino acids (e.g. incorporation into protein) induced by the BCAAs (Louard, 1990; Schwenk, 1987).

In conclusion, administration of BCAAs to ALS patients did not effect the glutamate metabolism but rather seemed to alter the protein metabolism in the muscles.

Acknowledgement

This work was supported by grants from P. Carl Petersens Foundation, Danish Hospital Foundation for Medical Research, Foundation for experimental research in Neurology.

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Received May 15, 1995