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Soy Peptide Ingestion Increases Neuroactive Amino Acids in the Adult Brain of Wild-Type and Genetically Engineered Serine-Deficient Mice

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Abstract

The aim of this study was to assess the neurochemical effects produced by short-term dietary soy peptide ingestion in C57BL/6 wild-type mice and in serine-deficient mice that were created as a genetic serine-deficiency disease model. D, L-Amino acid analysis demonstrated that overnight oral ingestion of a 35% (w/v) soy peptide solution significantly increased the hippocampal tissue content of certain neuroactive amino acids in both genotype groups of mice. These amino acids included the neurotransmitter L-glutamate, its precursor L-glutamine, the neuromodulator D-aspartate, and branched-chain amino acids L-valine, L-leucine, and L-isoleucine. Soy peptide ingestion caused similar increases in contents of L-glutamine and branched chain amino acids in the cerebral cortex. Oral ingestion of a 150 mM L-serine solution did not alter contents of these amino acids in the hippocampus and the cerebral cortex in both genotype groups. The present findings indicate that the short-term oral intake of soy peptide positively modulates the levels of certain neuroactive amino acids in the adult brain.

Keywords: Branched-chain amino acids; D-aspartate; L-glutamate ; neuroactive amino acids; soy peptide.

Abbreviations: N-acetyl-L-cysteine, NAC; o-phthalaldehyde, OPA; D-3-phosphoglycerate dehydrogenase, Phgdh; water filtered by reverse osmotic membrane, ROW; reverse-phase high-performance liquid chromatography, RP-HPLC; genetic serine-deficiency disease model, SDDM.

Introduction

Dietary ingestion of soy proteins has proven beneficial for improving metabolic syndrome-related symptoms, e.g.atherosclerosis, fatty liver, dyslipidemia, insulin resistance, and obesity [1-3]. In addition to soy proteins, a recent study in humans reported that ingestion of a soy peptide mixture composed of di- and tri-peptides increased the serum levels of amino acids more rapidly than the original soy proteins or an amino acid mixture with an equivalent amino acid composition did [4]. Furthermore, ingestion of soy peptides produced beneficial outcomes in humans, which included alleviation of fatigue, promotion of relaxation, and modulation of electroencephalogram [5]. Although these outcomes suggest that the observed effects result from actions on the central or peripheral nervous system, the neurochemical effects of soy peptides remain largely unknown. Therefore, in the present study, we examined the effects of short-term soy peptide ingestion on the amino acid composition of the brains from wild-type mice and serine-deficient mice that were created as a genetic serine-deficiency disease model (SDDM). The SDDM mice have a brain-specific deletion of the gene encoding D-3-phosphoglycerate dehydrogenase (Phgdh) (EC 1.1.1.95), which catalyzes the formation of 3-phosphohydroxypyruvate from 3-phosphoglycerate, the first step in *de novo* biosynthesis of L-serine [6]. Brain-specific deletion of this gene results in marked decreases in L- and D-serine and mild microcephaly [7], which resembles the impairments observed in patients with genetic serine-deficiency disease [8].

Materials and Methods

Animal experiments

In this study, the animal experiments were conducted in accordance with the "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain" (Notice no. 88, Ministry of the Environment, Government of Japan). All animal protocols were approved by the Animal Ethics Committee of Kyushu University. SDDM mice with brain-specific deletion of *Phgdh* (Tg (*GFAP*-Cre) 25Mes/0, $Phgdh^{tm1.2Shfu}/Phgdh^{tm1.2Shfu}$) were generated and maintained in a pathogen–free animal facility with controlled temperature and lighting conditions, as described previously [7]. C57BL/6J mice were obtained from Kyudo Co. (Saga, Japan). Female SDDM mice at 10–13 weeks of age and female C57BL/6J mice at 11–12 weeks of age were transferred from their breeding facility to the animal quarters at the laboratory and housed individually in wire cages in an air-conditioned environment (22 ± 3°C) with a 12-h light/12-h dark cycle. During the first 30 h after transfer the mice were given water and standard laboratory chow ad libitum, and then they were fasted for 6 h (from 18:00 to 24:00). At 0:00 h on the day of the experiment, mice were sorted into three groups, and each group was allowed free access to one of three test solutions: water filtered by a reverse osmotic membrane (ROW), a soy peptide solution (35% (w/v) in ROW), or an L-serine solution (150 mM in ROW). The soy peptide solution used in this study was Hinute-AM, which was composed primarily of di- and tri-peptides [9] and was kindly supplied by Fuji Oil Co. (Osaka, Japan). The concentration of L-serine in the serine solution approximated the concentration of L-serine in 11S globulin, a major soy protein source of Hinute-AM,

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at a concentration of 35% (w/v). Mice were given free access to each solution for 8 h and then were sacrificed under diethyl ether anesthesia. The brains of SDDM mice and C57BL/6J wild-type mice were rapidly removed, and each was placed onto an ice-cold glass plate. Each hippocampus and cerebrum was isolated, weighed, and stored at −80°C. For C57BL/6J wild-type mice, blood and liver samples also were collected and stored at −80°C.

D,L-Amino acid analysis

The D- and L-amino acid contents of tissue and serum samples were determined using a reverse-phase high-performance liquid chromatography (RP-HPLC) (ACQUITY UPLC TUV system, Waters, Milford, MA, USA) after derivatization of free amino acids with o-phthalaldehyde (OPA) and N-acetyl-L-cysteine (NAC), as described previously [10]. In this system, concentrations of alanine, glycine, γ-aminobutyric acid, threonine, and tryptophan were not determined because of limited separation. The D-amino acid content in the soy peptide preparation, 500 μg of Hinute-AM, was determined by hydrolyzing the solution in 6 M HCl solution that contained 1% phenol at 110°C for 6, 15, or 20 h. The resultant hydrolysates were dissolved in 100 μl of Milli-Q grade water and passed through a LG filter (0.2 μm-cut) (Millipore, Billerica MA, USA). The filtered samples were derivatized with OPA and NAC or OPA and N-tert-butoxycarbonyl-L-cysteine, as described [11,12]. The D-amino acid content was then determined using the RP-HPLC system as described above.

Statistical analysis

The differences in amino acid concentrations among the treatment groups were analyzed using a one-way ANOVA followed by Dunnet test. P-values ≤ 0.05 were considered significantly different. All statistical tests were performed using KaleidaGraph, version 4.0 (Synergy Software, Reading, PA, USA).

Results and Discussion

Ingestion of soy peptides or L-serine caused significant changes in the concentrations of several amino acids in the hippocampi of SDDM and wild-type mice (Table 1). As expected, L-serine ingestion produced a marked increase in free L-serine concentration in the hippocampi of SDDM and C57BL/6J mice when compared with those of mice that ingested ROW or soy peptides. L-Serine ingestion also produced a marked increase in D-serine concentration in the hippocampus of C57BL/6J, suggesting that L-serine was converted to D-serine after absorption from the intestine. Unlike L-serine ingestion, soy peptide ingestion affected neither the L- nor D-serine concentration in the hippocampi of SDDM and C57BL/6J mice. However, soy peptide ingestion did produce distinct changes in the concentrations of certain neuroactive amino acids in the hippocampus. Among the amino acids examined, concentrations of L-glutamate and L-glutamine increased significantly in SDDM and C57BL/6J mice after soy peptide ingestion when compared with those of mice that had ingested ROW or L-serine. Furthermore, soy

Amino acid		Treatment			Significance	
	ROW	SOY	SER	ROW vs SOY	ROW vs SER	SOY vs SER
SDDM						
L-Serine	0.0751 ± 0.0054	0.0960 ± 0.0057	0.379 ± 0.103	*N.S.*	*p < 0.01*	*p < 0.05*
D-Serine	0.0220 ± 0.0037	0.0191 ± 0.0012	0.0915 ± 0.0374	*N.S.*	*N.S.*	*p = 0.10*
L-Glutamate	4.095 ± 0.507	5.765 ± 0.195	4.924 ± 0.403	*p < 0.05*	*N.S.*	*N.S.*
L-Glutamine	2.904 ± 0.285	5.091 ± 0.248	3.476 ± 0.173	*p < 0.0005*	*N.S.*	*p < 0.005*
L-Aspartate	1.813 ± 0.185	2.606 ± 0.113	1.971 ± 0.232	*p = 0.07*	*N.S.*	*N.S.*
L-Valine	0.0568 ± 0.0086	0.105 ± 0.005	0.0695 ± 0.0092	*p < 0.0005*	*N.S.*	*p < 0.005*
L-Leucine	0.0695 ± 0.0094	0.120 ± 0.005	0.0840 ± 0.0110	*p < 0.01*	*N.S.*	*p < 0.05*
L-Isoleucine	0.0189 ± 0.0033	0.0351 ± 0.0020	0.0246 ± 0.0039	*p < 0.05*	*N.S.*	*N.S.*
C57BL/6J						
L-Serine	0.367 ± 0.005	0.378 ± 0.010	0.533 ± 0.064	*N.S.*	*p < 0.05*	*p < 0.05*
D-Serine	0.140 ± 0.003	0.136 ± 0.003	0.154 ± 0.007	*N.S.*	*N.S.*	*p < 0.05*
L-Glutamate	3.928 ± 0.429	4.487 ± 0.195	3.835 ± 0.403	*p < 0.05*	*N.S.*	*p < 0.005*
L-Glutamine	2.654 ± 0.122	4.267 ± 0.656	2.752 ± 0.226	*p = 0.09*	*N.S.*	*p = 0.09*
L-Aspartate	1.344 ± 0.185	1.544 ± 0.113	1.500 ± 0.232	*N.S.*	*N.S.*	*N.S.*
L-Valine	0.0450 ± 0.0018	0.0769 ± 0.0047	0.0469 ± 0.0017	*p = 0.0001*	*N.S.*	*p = 0.001*
L-Leucine	0.0671 ± 0.0035	0.0844 ± 0.0028	0.0687 ± 0.0051	*p < 0.05*	*N.S.*	*p < 0.05*
L-Isoleucine	0.0177 ± 0.0010	0.0230 ± 0.0009	0.0182 ± 0.0010	*p < 0.01*	*N.S.*	*p < 0.01*

Abbreviations: ROW, water filtered by reverse osmotic membrane (control); SER, L-serine; SOY, soy peptides;
SDDM, genetic serine-deficiency disease model; N.S., not significant.
Values are means ± standard errors (SDDM mice: n = 5 for the ROW group; n = 4 for SOY group; n = 4 for the SER group;
C57BL/6J mice: n = 4 for the ROW group; n = 6 for the SOY group; n = 5 for the SER group).
Amio acid contents in the tissue are shown in nmol/mg wet tissue.

Table 1: Effects of soy peptide or L-serine ingestion on amino acid concentrations in hippocampi of SDDM and C57BL/6J mice.

peptide ingestion produced ~1.7-fold increases in the concentrations of the branched-chain amino acids (BCAA) L-valine, L-leucine, and L-isoleucine in the hippocampus of SDDM mice. Significant (1.3~1.7-fold) increases in the concentrations of BCAA also were observed in the hippocampus of C57BL/6J. When the amino acid content of the cerebral cortex was examined, soy peptide ingestion by SDDM and C57BL/6J mice produced significant increases in the concentrations of L-glutamine, L-valine, L-leucine, and L-isoleucine when compared with these amino acids in the cerebral cortex of ROW group (Table 2). L-Serine ingestion produced a significant increase in only the concentration of L-serine in the cerebral cortex when compared with the amino acids in the cerebral cortex of ROW group (Table 2). In addition to these major L-amino acids, we found a significant increase in the content of an unusual minor amino acid D-aspartate in the hippocampi of both types of mice (Figure 1). A clear trend toward increasing D-aspartate content was also seen in the cerebral cortex of SDDM (0.0360 ± 0.0014 nmol/mg tissue: $p < 0.05$) and C57BL/6J (0.0337 ± 0.0005 nmol/mg tissue: $p = 0.08$) mice when compared with ROW group (0.0300 ± 0.0015 nmol/mg tissue for SDDM, 0.0308 ± 0.0019 nmol/mg tissue for C57BL/6J).

To ascertain whether the concentrations of free amino acids in other tissues were altered by ingestion of soy peptides or

Amino acid		Treatment			Significance	
	ROW	SOY	SER	ROW vs SOY	ROW vs SER	SOY vs SER
SDDM						
L-Serine	0.0783 ± 0.0092	0.100 ± 0.022	0.346 ± 0.079	*N.S.*	*p < 0.001*	*p < 0.005*
D-Serine	0.0227 ± 0.0043	0.0284 ± 0.0012	0.0814 ± 0.0290	*N.S.*	*p < 0.05*	*p = 0.06*
L-Glutamate	4.132 ± 0.136	4.394 ± 0.137	4.276 ± 0.103	*N.S.*	*N.S.*	*N.S.*
L-Glutamine	2.965 ± 0.123	3.808 ± 0.224	3.138 ± 0.196	*p < 0.01*	*N.S.*	*p = 0.05*
L-Aspartate	1.934 ± 0.101	2.000 ± 0.121	1.975 ± 0.160	*N.S.*	*N.S.*	*N.S.*
L-Valine	0.0631 ± 0.0032	0.0815 ± 0.0032	0.0676 ± 0.0028	*p < 0.005*	*N.S.*	*p < 0.05*
L-Leucine	0.0803 ± 0.0042	0.0993 ± 0.0036	0.0831 ± 0.0048	*p < 0.05*	*N.S.*	*p < 0.05*
L-Isoleucine	0.0223 ± 0.0014	0.0277 ± 0.0012	0.0236 ± 0.0013	*p < 0.05*	*N.S.*	*p = 0.10*
C57BL/6J						
L-Serine	0.340 ± 0.007	0.344 ± 0.013	0.472 ± 0.049	*N.S.*	*p < 0.05*	*p < 0.05*
D-Serine	0.138 ± 0.004	0.132 ± 0.004	0.140 ± 0.005	*N.S.*	*N.S.*	*N.S.*
L-Glutamate	3.609 ± 0.135	4.007 ± 0.132	3.260 ± 0.046	*p = 0.06*	*N.S.*	*p < 0.001*
L-Glutamine	2.507 ± 0.126	3.928 ± 0.493	2.525 ± 0.172	*p < 0.05*	*N.S.*	*p < 0.05*
L-Aspartate	1.675 ± 0.118	1.891 ± 0.096	1.632 ± 0.057	*N.S.*	*N.S.*	*N.S.*
L-Valine	0.0560 ± 0.0038	0.0840 ± 0.0050	0.0526 ± 0.0023	*p = 0.001*	*N.S.*	*p = 0.0005*
L-Leucine	0.0737 ± 0.0053	0.0855 ± 0.0032	0.0688 ± 0.0066	*N.S.*	*N.S.*	*p = 0.06*
L-Isoleucine	0.0189 ± 0.0011	0.0227 ± 0.0011	0.0173 ± 0.0013	*p = 0.08*	*N.S.*	*p < 0.05*

Abbreviations: ROW, water filtered by reverse osmotic membrane (control); SER, L-serine; SOY, soy peptides; SDDM, genetic serine-deficiency disease model; N.S., not significant. Values are means ± standard errors (SDDM mice: n = 5 for the ROW group; n = 4 for SOY group; n = 4 for the SER group; C57BL/6J mice: n = 4 for the ROW group; n = 6 for the SOY group; n = 5 for the SER group). Amio acid contents in the tissue are shown in nmol/mg wet tissue.

Table 2: Effects of soy peptide or L-serine ingestion on amino acid concentrations in cerebral cortices of SDDM and C57BL/6J mice.

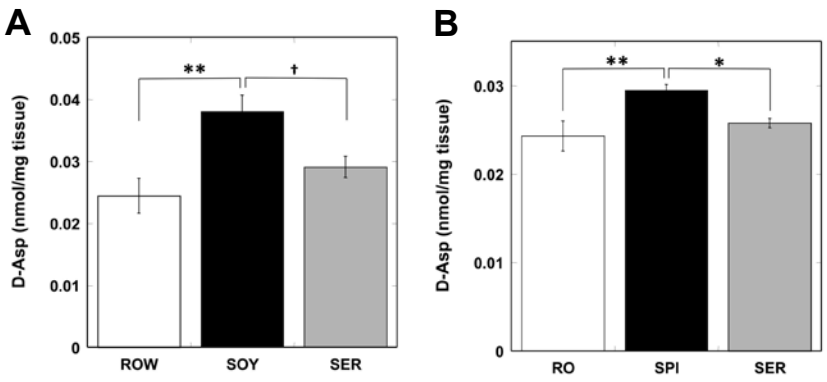

Figure 1: Increase in free D-aspartate level of the hippocampus after soy peptide ingestion. Hippocampal D-aspartate levels of SDDM (A) and C57BL/6J wild type (B) mice were measured as described in the Materials and Methods. Data represent the mean amino acid content ± S.E. (SDDM mice: n = 5 for the ROW group; n = 4 for SOY group; n = 4 for the SER group; C57BL/6J mice: n = 4 for the ROW group; n = 6 for the SOY group; n = 5 for the SER group). Differences between treatments were analyzed by one-way ANOVA, Dunnett's post-hoc test (*, p < 0.05; **, p < 0.005; † indicates p = 0.06).

L-serine, we determined their concentrations in the liver tissues and serum from C57BL/6J mice. Soy peptide ingestion significantly increased liver concentrations of D-aspartic acid, L-aspartic acid, L-valine, L-leucine, L-isoleucine, and L-tyrosine (Table 3). L-Serine ingestion produced a statistically significant increase in D-serine concentration in only the livers from C57BL/6J mice. Soy peptide ingestion significantly elevated serum concentrations of L-valine, L-leucine, L-isoleucine, L-arginine, L-histidine, L-phenylalanine, and L-tyrosine. Soy peptide ingestion produced a non-significant increase in serum L-aspartate concentration when compared with its concentration in sera from mice that had ingested ROW (p = 0.07). D-Aspartate was not detected in serum samples. L-Serine ingestion did not alter serum concentrations of any of the amino acids.

An unexpected finding of the present study was that short-term ingestion of soy peptides resulted in a significant increase in the concentration of D-aspartate in the hippocampus and the liver (Figure 1 and Table 3). This is the first time that ingestion of a botanical protein-derived material has been shown to be capable of elevating the tissue content of D-aspartate in the brain. It is well documented that peptidyl D-amino acids occur naturally in peptides and proteins in various organisms, including mammals. Indeed, D-aspartate residues are found at specific sites in α-A-crystallin, a major structural protein of the eye lens [13]. To gain insight into the mechanism associated with the elevation of D-aspartate by soy peptide ingestion, we assayed for D-aspartate in the soy peptide preparation. We measured D-aspartate and other D-amino acid concentrations in acid-hydrolyzed soy peptide samples as a function of time. D-Aspartate was detected in the hydrolysates, and the D: (D + L) ratio of aspartate increased linearly with hydrolysis time (Table 4). When we extrapolated to estimate concentrations at time 0, the relative content of D-aspartate was approximately 3.5% of total aspartate and asparagine content in the soy peptide preparation. D-Alanine, D-valine, and D-glutamate also were present in the acid lysate. Thus, it is likely that the soy peptide preparation used in this study contained D-amino acids. However, we cannot exclude the possibility that these D-amino acids were generated artificially during acid hydrolysis of the peptides. Indeed, we did not detect these D-amino acids except for D-aspartate in sera and liver samples from mice that had ingested soy peptides. Thus, further biochemical analysis will be needed to prove the occurrence of D-amino acids in the soy peptide preparation.

The initial goal of the present study was to test whether soy peptide ingestion increases L- and D-serine concentrations in the brains of SDDM mice and to compare the effects of soy peptide ingestion with those of L-serine ingestion. Patients with serine-deficiency disease have lower free serine enantiomer levels in plasma and cerebrospinal fluid that are most likely caused by harmful genetic mutations in enzymes involved in the *de novo* synthesis of L-serine. These patients exhibit severe neurological symptoms, including congenital microcephaly, psychomotor retardation, and intractable seizures. Daily oral supplementation with a relatively high dose of L-serine restores concentrations of L- and D-serine to a normal healthy range [14,15]. If

Amino acid		Treatment			Significance	
Liver (nmol/mg wet tissue)	ROW	SOY	SER	ROW vs SOY	ROW vs SER	SOY vs SER
D-Serine	0.00383 ± 0.00031	0.00975 ± 0.00069	0.0223 ± 0.0068	*N.S.*	*p < 0.05*	*p = 0.09*
D-Aspartate	0.0176 ± 0.0024	0.0347 ± 0.0033	0.0181 ± 0.0020	*p < 0.005*	*N.S.*	*p < 0.005*
L-Aspartate	0.148 ± 0.015	0.265 ± 0.039	0.167 ± 0.015	*p < 0.05*	*N.S.*	*p = 0.08*
L-Valine	0.246 ± 0.007	0.437 ± 0.038	0.255 ± 0.011	*p < 0.005*	*N.S.*	*p < 0.005*
L-Leucine	0.479 ± 0.017	0.616 ± 0.043	0.516 ± 0.016	*p < 0.05*	*N.S.*	*p = 0.09*
L-Isoleucine	0.104 ± 0.002	0.148 ± 0.010	0.113 ± 0.005	*p < 0.01*	*N.S.*	*p < 0.05*
L-Tyrosine	0.104 ± 0.002	0.148 ± 0.010	0.113 ± 0.005	*p < 0.01*	*N.S.*	*p < 0.05*
Serum (mM)						
L-Serine	0.101 ± 0.004	0.226 ± 0.026	0.781 ± 0.477	*N.S.*	*N.S.*	*N.S.*
L-Glutamate	0.128 ± 0.021	0.198 ± 0.024	0.155 ± 0.020	*p = 0.11*	*N.S.*	*N.S.*
L-Glutamine	2.654 ± 0.122	4.267 ± 0.656	2.752 ± 0.226	*p = 0.09*	*N.S.*	*p = 0.09*
L-Aspartate	0.0252 ± 0.0081	0.0641 ± 0.0119	0.0403 ± 0.0103	*p = 0.07*	*N.S.*	*N.S.*
L-Valine	0.132 ± 0.010	0.412 ± 0.060	0.135 ± 0.011	*p < 0.005*	*N.S.*	*p < 0.005*
L-Leucine	0.161 ± 0.011	0.398 ± 0.064	0.178 ± 0.023	*p < 0.05*	*N.S.*	*p < 0.05*
L-Isoleucine	0.0513 ± 0.0040	0.137 ± 0.023	0.0583 ± 0.0052	*p < 0.01*	*N.S.*	*p < 0.05*
L-Arginine	0.0921± 0.0106	0.211 ± 0.039	0.127 ± 0.020	*p < 0.05*	*N.S.*	*N.S.*
L-Histidine	0.0498 ± 0.0018	0.0789 ± 0.0063	0.0553 ± 0.0031	*p < 0.005*	*N.S.*	*p < 0.01*
L-Phenylalanine	0.0829 ± 0.0056	0.141 ± 0.020	0.0984 ± 0.0058	*p < 0.05*	*N.S.*	*N.S.*
L-Tyrosine	0.0638 ± 0.0068	0.182 ± 0.036	0.0776 ± 0.0040	*p < 0.05*	*N.S.*	*p < 0.05*

Abbreviations: ROW, water filtered by reverse osmotic membrane (control); SER, L-serine; SOY, soy peptides; SDDM, genetic serine-deficiency disease model; N.S., not significant. Values are means ± standard errors (n = 4 for ROW group, n = 6 for SOY group, n = 5 for SER group). Amio acid contents in the liver are shown in nmol/mg wet tissue. Amio acid concentrations in serum are shown in mM.

Table 3: Effect of the ingestion of soy peptide or L-serine on amino acid concentrations in the liver and serum of C57BL/6J mice.

		Hydrolyzation		
Amino acids	6 h	15 h	20 h	0 h*
Aspartate	4.672 ± 0.178	6.544 ± 0.455	7.458 ± 0.432	3.491
Alanine	0.620 ± 0.029	0.985 ± 0.215	1.263 ± 0.117	0.337
Valine	1.179 ± 0.283	2.532 ± 0.301	2.668 ± 0.428	0.601
Glutamate	1.327 ± 0.110	2.119 ± 0.156	2.413 ± 0.126	0.876

Values (%) represent means with standard deviations (n = 3).
*Values at 0 h were estimated.
Soy peptide mixture was hydrolyzed in in 6 M HCl solution containing 1% phenol at 110ºC for the indicated hydrolysis times.

Table 4: The D/D+L ratio of amino acids in soy peptide hydrolysate.

brain L- and D-serine levels are enhanced efficiently by ingestion of soy peptides, which is a widely used food supplement, one might expect that ingestion of soy peptides could provide an additional nutritional treatment to improve patients' neurological symptoms. However, soy peptide ingestion was not effective in increasing L- and D-serine concentrations in the brain (Tables 1 and 2). Instead, we found that soy peptide ingestion significantly increased the concentration of L-glutamate, L-glutamine, D-aspartate, and BCAA in the hippocampus (Table 1). A similar trend of increases in these amino acids was also seen in the cerebral cortex (Table 2).

L-Glutamate is the major excitatory neurotransmitter in the mammalian brain and L-glutamine serves as its precursor in neurons via the glutamate-glutamine cycle [16]. Impaired or reduced glutamatergic neurotransmission is related to the development of a wide variety of neurological diseases, e.g., schizophrenia, a psychiatric disorder involving hypofunction of N-Methyl-D-aspartic acid (NMDA)-selective glutamate receptor-mediated neurotransmission [17]. Given that there is a little passage of L-glutamate or L-glutamine across the blood-brain barrier [18], the mechanism by which soy peptide ingestion modulates brain levels of these amino acids is unknown, but intake of soy peptides could contribute to the maintenance of L-glutamate and its precursor L-glutamine in the brain.

A significant increase in D-aspartate level and non-significant increase in L-aspartate level also were observed in the hippocampi and the cerebral cortices of mice that ingested soy peptides (Figure 1, Tables 1 and 2). A recent study has demonstrated that D-aspartate is generated from L-aspartate in mammalian brains by aspartate racemase, which is also expressed in the heart, adrenal cortex, and testis but not in the liver, lung, or kidney [19]. Because D-aspartate was not detected in serum samples from the mice that ingested soy peptides, it seems likely that D-aspartate in brain was generated enzymatically from L-aspartate by aspartate racemase. On the other hand, increased level of D-aspartate in the liver likely originated from the peptidyl D-aspartate which presents in the soy peptide preparation itself (Tables 3 and 4). It was also shown by Kim et al. [19] that shRNA knockdown of aspartate racemase results in impaired dendritic growth and reduced survival of new neurons in the adult hippocampus, suggesting a role for D-aspartate in the neurogenesis in the adult hippocampus.

Additionally, soy peptide ingestion produced marked elevations in BCAA concentrations in brain, liver, and serum (Tables 1 - 3). This may reflect the fact that soy protein and peptide have the highest protein digestibility-corrected amino acid score [20]. BCAA are transported efficiently across the blood-brain barrier and have a unique role in brain neurotransmitter metabolism. Sakai et al. [21] used [^{15}N] - and [U-^{13}C]-leucine to show that at least 50% of the L-glutamate in rat brain was derived from L-leucine. Thus, it appears possible that L-leucine derived from the soy peptide preparation contributed to the increased level of L-glutamate in the brains of mice in our study. In addition to effects on neurotransmitter metabolism, a recent study demonstrated that dietary BCAA supplementation promoted cognitive improvement by restoring hippocampal synaptic function after traumatic brain injury [22].

In conclusion, short-term ingestion of soy peptides produced exceptional effects on amino acid concentrations in the adult mouse brain, which suggests that soy peptides may be beneficial in maintaining brain function and in treating brain damage. Additional animal studies aimed at examining the effects of chronic ingestion of soy peptides on brain physiology and pathology will illuminate how soy peptides facilitate healthy brain function. Additional clinical studies are also needed to expand the potential applications of soy peptides as an effective nutraceutical for promoting brain health.

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