Comparison of the Effect of Soy and Casein-Derived Peptide Administration on Tyrosine and Catecholamine Metabolism in the Mouse Brain

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(Received January 17, 2018)

Summary The effect of soy and casein peptide intake on the metabolism of amino acids and monoamine neurotransmitters in the serum and brain were examined in C57BL/6 mice. Acute oral administration of soy peptide (0.026 g/30 g body weight) caused a notable increase in tyrosine, a catecholamine precursor, in the serum and cerebral cortex, whereas casein peptide administration at the same dose led to an increase in tyrosine in the serum, but not in the cerebral cortex. In addition to tyrosine, soy peptide administration also led to an effective augmentation of 3-methoxy-4-hydroxyphenylethylenglycol (MHPG), a principal metabolite of noradrenaline, and significant facilitation of noradrenergic turnover in the cerebral cortex, brainstem, and hippocampus compared to the vehicle control. Casein peptide administration also led to an increase in MHPG only in the cerebral cortex, and caused facilitation of noradrenergic turnover in the cerebral cortex and brainstem. These in vivo observations suggest that both soy and casein peptide intake at this concentration can lead to an increased availability of tyrosine and stimulation of noradrenergic turnover in the brain.

Key Words soy, casein, tyrosine, brain, noradrenalin

It has been demonstrated that soy products exert various beneficial effects on human health, and it is anticipated that dietary intake of soy products might contribute to the longevity or health promotion of older adults in East Asian countries (1). Soy proteins and their hydrolyzed peptides have been shown to elicit various biological effects such as exhibiting anti-cancer, anti-oxidative, anti-hypertensive, and anti-obesity activities in vivo (2). Further, oral intake of soy peptide improved the barrier function of skin (3) and reduced muscle strain (4, 5). Soy peptides composed mainly of di- and tripeptides, which have high absorbability (6), improved hippocampus-dependent spatial memory function in senescence-accelerated mice (7) and the recognition memory performance of healthy young human volunteers (8). Although these favorable effects have been mentioned, the effect of soy peptide intake on brain metabolism and function is yet to be fully elucidated.

Our in vivo studies showed that oral intake of soy peptides via drinking water increased L-Glu and branched-chain amino acid (BCAA) levels in the cerebral cortex and hippocampus (9). In addition to these amino acids, a significant increase in L-Tyr was observed in the serum. Furthermore, we recently demonstrated that the L-Ser-L-Tyr (SY) dipeptide sequence appears more frequently in several soy proteins than other L-Tyr-containing dipeptide sequences, and oral administration of its synthetic dipeptide led to a marked increase in L-Tyr content in the mouse brain more efficiently than those of the other two L-Tyr-containing synthetic dipeptides or sole L-Tyr at the same intake dose (10). Noradrenaline (NA), also known as norepinephrine, serves as a catecholamine neurotransmitter in the brain, and is synthesized from L-Tyr. In parallel with an increase in L-Tyr, acute oral administration of SY elicited a significantly higher noradrenergic turnover than other L-Tyr content dipeptides or L-Tyr alone administration in the mouse brain (11). Moreover, our recent study of mice demonstrated that oral intake of soy peptide at a higher dose (0.26 g/0.6 mL/30 g body weight) markedly increased L-Tyr level in the serum, which was accompanied by accelerated brain NA metabolism (12).

Milk casein-derived peptides also have been shown to exhibit biological activities that potentially contribute to health-related quality of life and alleviate symptoms of various chronic human diseases in some cases (13–15). As in soy peptides, it was reported that oral intake of casein-hydrolyzed peptides increased Tyr and BCAA in the human plasma more efficiently than casein protein (16). However, it is still not well understood whether casein peptide ingestion affects the neurotransmitter...
metabolism in the brain. In this study, we sought to compare the effect of the oral intake of soy peptide with casein peptide at a lower dose on L-Tyr and catecholamine neurotransmitter metabolism in the mouse brain.

MATERIALS AND METHODS

Materials. A soy peptide mixture, Hinite-AM, was provided by Fuji Oil Co. Ltd. (Osaka, Japan). A casein peptide mixture, Peptopro, was obtained from DSM Food Specialties (Delf, The Netherlands). These commercially available peptide products are composed primarily of di- and tripeptides. Adrenaline (also known as epinephrine), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5-HIAA), 5-hydroxytryptamine (5-HT), homovanillic acid (HVA), 3-methoxytyramine (3-MT), NA, normetanephrine (NM), and 3-methoxy-4-hydroxyphenethylamine (MHPG) were obtained from Sigma-Aldrich (St. Louis, MO).

Animal experiments. All animal experiments in this study followed 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 experiments and procedures were reviewed and approved by the Animal Experiment Committee of Kyushu University (Permit Number: A25-121 and A27-103). Male C57BL/6N mice at 8 wk of age were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan). They were maintained at the Biotron Institute of Kyushu University on a 12 h light/12 h dark cycle (lights on 8 a.m. to 8 p.m.) in an air-conditioned room (25 ± 1°C) with 60% humidity under specific pathogen-free conditions. They were then transferred to the breeding room at the laboratory in a controlled environment described above and kept overnight with access to water and standard laboratory chow. After one night, mice were divided into three groups, and a mouse was considered significantly different. All statistical tests were carried out using KaleidaGraph, version 4.5 (Synergy Software, Reading, PA).

RESULTS

Firstly, we determined the levels of amino acids in the serum and cerebral cortex of peptide-treated and vehicle groups. Acute soy or casein peptide administration led to an increase of L-Leu, which is one of the BCAAs, in the serum (Fig. 1A). Concentrations of L-Leu were 1.59-fold and 2.07-fold higher in the soy peptide-treated group and the casein one, respectively, than in the vehicle group. However, the contents of L-Leu in the cerebral cortex remained unchanged after either soy or casein peptide administration (Supplemental Online Material, Tables S1 and S2). As in L-Leu and L-Ile, soy and casein peptide administration led to similar 2.85- and 2.99-fold increases in serum L-Tyr, respectively, compared to that of the vehicle group (Fig. 2A). However, the contents of L-Tyr in the cerebral cortex were 2.33-fold and 1.61-fold higher in the soy peptide-treated group and casein group, respectively, than in the vehicle group (Fig. 2B). Although the increase in serum...
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The l-Tyr concentration of the soy and casein groups was similar in magnitude, soy peptide led to a more notable increase in the cerebral l-Tyr content than casein peptide 30 min after administration. In addition to these amino acids, l-Ala concentration also significantly rose in the serum of the soy peptide-treated group, and an increase in l-Met and a decrease in Gly concentrations were observed in the serum of the casein-peptide treated group (Supplemental Online Material, Table S1). In the cerebral cortex, a subtle but significant decrease in d-Asp content in the soy peptide-treated group, and significant decreases in d-Ser, l-Ala and l-Phe contents in the casein peptide-treated group were detected (Supplemental Online Material, Table S2).

Next we determined levels of neurotransmitter monoamines and their metabolites in the cerebral cortex, brainstem, and hippocampus following the peptide or vehicle administration. NA is synthesized from l-Tyr principally in neurons of the locus ceruleus in the brainstem. Although NA levels did not alter in either group, the levels of MHPG, a predominant metabolite of NA in the brain, were 1.60-fold and 1.46-fold higher in the soy peptide-treated group and the casein-peptide group, respectively, in the cerebral cortex (Fig. 3A). In the brainstem, MHPG levels were 1.40-fold greater in the soy peptide-treated group than that in the vehicle group, whereas no significant change was seen between the casein peptide-treated and vehicle groups (Fig. 3B). A significant 1.50-fold increase in MHPG content was detected also in the hippocampus (Fig. 3C). When examining the effect on dopamine (DA) and their metabolites, we found that only the homovanillic acid (HVA) level significantly increased to 1.29-fold in the cerebral cortex of the soy peptide-treated group compared to that of the vehicle group (Supplemental Online Material, Table S3). There were no significant changes in DA or its metabo-
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In the brainstem or hippocampus among groups (Supplemental Online Material, Tables S4 and S5). Although serotonin (5-HT) and its metabolite levels did not change in the cerebral cortex or brainstem among the groups examined (Supplemental Online Material, Tables S3 and S4), a significant 0.73-fold decrease in 5-HT was detected in the hippocampus (Supplemental Online Material, Table S5).

We then evaluated monoamine turnover in the cerebral cortex and brainstem. Noradrenergic turnover, as determined by the ratio of MHPG and NM to NA serves as the index of neurotransmission of NA. In the cerebral cortex, noradrenergic turnover of the soy peptide- and casein peptide-treated groups showed significant 1.36-fold and 1.21-fold increases, respectively, compared with that of the vehicle group. It is of note that the soy peptide-treated group exhibited a higher turnover rate than did the casein group ($p=0.0816$) (Fig. 4A). In the brainstem, noradrenergic turnover of the soy peptide- and the casein peptide-treated groups increased 1.37-fold and 1.24-fold compared to that of the vehicle group, although there was no significant difference between the
soy peptide- and casein peptide-treated groups (Fig. 4B). Noradrenergic turnover in the hippocampus of the soy peptide-treated group showed a trend toward increase compared to that of the vehicle group (p = 0.052, Fig. 4C). Dopaminergic turnover (DOPAC + HVA + 3-MT)/DA and 5-HT turnover (5-HIAA/5-HT) did not differ among the three groups in the cerebral cortex, hippocampus, or brainstem.

**DISCUSSION**

Peptides derived from food proteins exhibit various regulatory activities in peripheral tissues and the blood circulating system, raising expectations that some food-derived peptides may have beneficial effects on preventing chronic and life-style related diseases. However, our knowledge of how food proteins/peptides affect our brain metabolism is still limited. Our recent study demonstrated that acute oral intake of soy peptide at a high dose (0.26 g/30 g body weight equivalent to 433 g/50 kg body weight) elicited the stimulation of NA synthesis and metabolism in the cerebral cortex, hippocampus and brainstem, which were simultaneously associated with a marked increase in L-Tyr content in the brain (12). However, when considering the convenience of incorporating such ingredients beneficial for brain health, it is practically unrealistic if soy peptide can only exhibit such favorable effects on the brain at higher doses. NA has been shown to play a protective role against β-amyloid-induced neurotoxicity (17). Furthermore, it was demonstrated that NA suppresses inflammation of microglia cells in the brain by regulating gene expression (18). Therefore, in the present study we sought to examine the effect of soy peptide administration on brain L-Tyr and NA metabolism in the mouse brain. In addition to soy peptide, our preliminary study demonstrated that acute oral intake of casein peptide also increased L-Tyr in the serum as observed in the human study (Koyanagi et al., unpublished observation). Thus, the purpose of the present study was to compare the effect of soy peptide with that of casein peptide on brain L-Tyr and catecholamine metabolism at a lower intake dose.

As in the case of a high intake dose (12), the present study shows that at one-tenth of the above dose, soy peptide administration elicits significant increases in L-Tyr in the serum and cerebral cortex 30 min after the administration (Fig. 2), which simultaneously lead to an up-regulation of MHPG levels and noradrenergic turnover in the cerebral cortex, brainstem, and hippocampus (Figs. 3 and 4). However, there are some notable differences in effects on brain amino acids between the oral administration of high- and low-intake doses of soy peptide. We observed that the contents of L-Glu, L-Ile, L-Leu, and L-Val significantly rose in the cerebral cortex 30 min after the administration at a high dose (12), whereas the administration at a low dose failed to cause such changes in the contents of these amino acids in the cerebral cortex (Supplemental Online Material, Table S2). It is of interest that the low-dose administration of soy peptide resulted in marked increases in L-Ile and L-Leu in the serum (Fig. 1 and Supplemental Online Material, Table S1). Given that BCAA and aromatic amino acids share transporters at the blood-brain barrier (BBB), these observations raise the possibility that soy peptide-derived L-Tyr has yet-unknown biochemical characteristics, which can allow it to preferentially enter the brain from serum via the BBB. Furthermore, the oral administration of soy and casein peptides led to distinct changes in the amino acid profile of the serum: the concentration of Met in the serum of the casein peptide group was significantly higher than those of the vehicle and soy peptide groups, while the concentration of Ala in the serum of the soy peptide group was higher than that of the vehicle group (Supplemental Online Material, Table S1). Although a precise mechanism involved in these changes remains unexplored, it seems likely that the amino acid composition of the protein sources of these peptides may affect the amino acid profile of the serum after the oral intake. Indeed, according to Standard Tables of Food Composition in Japan 2015 (Seventh Revised Edition) by the Ministry of Education, Culture, Sports, Science and Technology, Japan (http://www.mext.go.jp/en/policy/science_technology/policy/title01/detail01/sdetail01/1388553.htm), casein proteins, the protein sources of the casein peptide used in this study, have a higher Met content (31 mg/g protein) than soy protein isolate (SPI) (14 mg/g protein), the protein source of the soy peptide used in this study. On the other hand, SPI shows a higher Ala content (44 mg/g protein) than casein proteins (31 mg/g protein). Tyr content in casein proteins and SPI are relatively higher than other amino acids and are estimated to be 57 mg/g protein and 42 mg/g protein, respectively (19).

In addition to soy peptide, the present study demonstrated for the first time that oral intake of casein peptide led to the elevation of L-Tyr levels in the serum to a similar extent (Fig. 2A). Although L-Tyr content in the cerebral cortex after casein peptide administration showed significantly lower levels than that of soy peptide, MHPG levels in the cerebral cortex and noradrenergic turnover in the cerebral cortex and brainstem were significantly enhanced as in soy peptide (Figs. 3 and 4). In addition to the effect on brain L-Tyr content, noradrenergic turnover facilitation in the cerebral cortex was less affected by casein peptide compared to soy peptide (Fig. 4A). However, molecular mechanisms underlying the observed differences in effects on brain L-Tyr availability and noradrenergic turnover caused by soy and casein peptides remain largely elusive. It is interesting to point out that L-Ser-L-Tyr (SY) is the most prevalent Tyr-containing dipeptide moiety in major soy proteins such as glycinin and beta-conglycinin (11), but is scarce in bovine caseins among surveyed sequences of di-peptide moieties containing Tyr residue in the Protein Database at National Center for Biotechnology Information (Maebuchi M, Ichinose T, Furuya S, unpublished observation). Our previous in vivo study demonstrated that synthetic SY is more potent in enhancing brain L-Tyr content and noradrenergic transmission than other synthetic dipeptides containing L-Tyr such as L-Ile-
l-Tyr and l-Tyr- l-Pro when administered orally (11). Indeed, the dipeptide SY was present in the soy peptide mixture used in this study, and its content was estimated to be at 2.18 ± 199 µg/g mixture (19). Thus, it is likely that the presence of SY is more prevalent in the soy peptide mixture than the casein peptide mixture, which may contribute to more efficient increases in l-Tyr availability and noradrenergic turnover in the brain.

Since noradrenaline reuptake inhibitors have been used as antidepressant drugs (20), it is likely that facilitation of noradrenergic transmission may prevent or delay the onset of depression. The present observations imply that l-Tyr-containing peptides derived from soy and casein proteins possess beneficial effects on brain noradrenergic transmission. Therefore, further studies are necessary for investigating the effect of long-term intake of these peptides on animal models of psychiatric diseases having dysregulation in NA synthesis and transmission.

Acknowledgments
This work was supported in part by funds from Fuji Oil Holdings Inc. and Fuji Foundation for Protein Research to S.F. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. We are grateful to Ms. HeeYung Woo for linguistic assistance.

Author contributions
Y.H., A.K., and S.F. designed the study. Y.H., A.K., M.M., and T.I. contributed to the acquisition of data, analysis, and interpretation of the data. Y.H. drafted the manuscript. Y.H. and S.F. critically revised the manuscript. All authors approved the final manuscript.

Supporting information
Supplemental Online Material is available on J-STAGE.

REFERENCES