Doctoral Thesis

Study on novel GABA-increasing dietary factors and their potential role in brain disease prevention

(Summary)

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学 位 論 文 の 要 旨

論文題目　Study on novel GABA-increasing dietary factors and their potential role in brain disease prevention
(新規 GABA 増加食餌因子と脳疾患予防におけるその潜在的役割に関する研究)

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1. Introduction (written in Chapter 1)

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter highly present in the brain. An imbalance in neurotransmission caused by either excessive glutamate-mediated excitation or insufficient GABA-mediated inhibition leads to various neurological diseases, including epilepsy. In order to correct this neurotransmission imbalance to prevent or treat those neurological diseases, GABA levels or GABA-inhibitory signals in the brain are needed to be increased. Aside from anticonvulsant drugs, such as vigabatrin (Vig), that are typically used to increase brain GABA levels, emerging research suggests that prebiotics and probiotics have a potential to ameliorate symptoms of neurological disorders by modulating gut microbiota that, in turn, release metabolites, including GABA, that act as mediators in restoring balance of GABA metabolism in the brain via increasing brain GABA levels itself or upregulating GABA receptors. Although it is widely accepted that prebiotics and probiotics can induce gut and brain GABA production via modulating gut microbiota, only evidence of probiotics has been so lidly demonstrated while the evidence of prebiotics is scarce. Moreover, there is no evidence showing the direct relationship among prebiotics, gut microbiota, gut GABA, and brain GABA.

Therefore, the objectives of this study were 1) to determine if dietary prebiotics have an ability in increasing peripheral and brain GABA levels, 2) to investigate if a typical prebiotic fructooligosaccharides (FOS) and its combination with the anti-epileptic drug Vig can elevate gut and brain GABA levels and ameliorate epilepsy, and 3) to explore endogenous brain functions of a brain-specific GABA-containing peptide, homocarnosine, which is a putative downstream mediator of GABA that its brain levels are newly found to be elevated by dietary prebiotics.

2. Effects of dietary factors on gut and brain GABA and gut microbiota composition

Chapter 2 investigated if the typical prebiotic FOS and newly proposed prebiotic *Aspergillus*-derived lipase (AL) and protease (AP) enzymes induces production of gut GABA and elevation of brain GABA by modulating gut microbiota. It was found that FOS, AP, and AL induced a trend increase in gut GABA levels and a significant elevation of brain GABA levels. Interestingly, this study is the first to demonstrate that prebiotics (FOS, AL, and AP) significantly increased a brain-specific GABA-containing peptide, homocarnosine, in the brain. A positive correlation between gut GABA levels and brain GABA or homocarnosine levels was observed under these prebiotic treatments. Bacterial genera that exhibited a positive correlation with gut and brain GABA/homocarnosine levels are *Parabacteroides*, *Akkermansia, Muribaculum, Hungatella*, *Marvinbryantia, Flavonifractor*, and *Incertae_sedis*. On the other hand, bacterial genera that exhibited a negative correlation with gut and brain GABA/homocarnosine levels are *Blautia*,

Unclassified_Lachnospiraceae, *Colidextribacter*, Acetatifactor, *Roseburia*, *Unclassified_Oscillospiraceae*, *Romboutsia*, and *Eubacterium_coprostanoligenes*. *Clostridium_sensu_stricto_1* and *Lachnospiraceae_A2* genera exhibited both positive and negative correlations with gut and brain GABA/homocarnosine levels. Taken together, prebiotics and prebiotic-like food factors mediate in the gut and brain connection by inducing gut GABA production and elevation of brain GABA and homocarnosine levels as a result of alteration in gut microbiota composition. These findings suggest a novel demonstration on the ability of dietary factors, other than probiotics, to induce brain GABA and homocarnosine production.

3. Adjunctive therapeutic potentials of FOS as a GABA-increasing dietary factor on epilepsy

Chapter 3 explored the adjunctive therapeutic potentials of FOS and its combination with the anti-epileptic drug Vig on pentylenetetrazol (PTZ)-induced epilepsy in mice. It was found that the combination of FOS and Vig exhibited synergistic effects on suppressing epileptic seizures and improving impaired cognitive function. Both FOS alone and the FOS + Vig combination increased cecal weight and gut GABA levels. In hippocampus, the Vig drug alone and the FOS + Vig combination significantly reduced a glutamic acid/GABA ratio and increased homocarnosine levels, in which the FOS + Vig combination exhibited a significantly stronger effect than the Vig drug alone in increasing homocarnosine levels. In cortex, a glutamic acid/GABA ratio was not changed across all groups, while an increase in homocarnosine levels was observed in the Vig drug alone group and the FOS + Vig combination group. FOS or the FOS + Vig combination upregulated antioxidant-defense genes (GPX2 and SOD2), downregulated an inflammatory marker (IL-6), and upregulated tight junction proteins (occludin and ZO-1) in hippocampus and/ or cortex. Taken together, FOS and its combination with the anti-epileptic drug Vig ameliorated epilepsy and improved cognitive function possibly through increasing gut and brain GABA and homocarnosine levels, increasing oxidative and anti-inflammatory defenses, and improved tight junctions of the brain.

4. Role of the endogenous GABA-containing peptide homocarnosine on brain functions

Chapter 2 and 3 demonstrated that prebiotics increased brain homocarnosine and this increased brain homocarnosine was found with lower seizure severity. Thus, in Chapter 4, potential roles of endogenous homocarnosine were explored using homocarnosine-deficient mice, carnosine synthase-1 (CARNS1) knockout (KO) mice. It was found that pre-aging (12 months of age) CARNS1-KO mice exhibited hyperactivity-, anxiety-, and depression-like behaviors, but had no defects in spontaneous locomotor activity, obsessive-compulsive behavior, olfactory functions, and learning and memory abilities, as compared with their age-matched wild-type mice. These findings provide new insights into roles of homocarnosine in mental health and behavior disorders.

5. General Conclusions (written in Chapter 5)

This body of work demonstrated the importance of dietary factors, particularly prebiotics, in brain health. Supplementation of prebiotics, such as FOS and *Aspergillus*-derived AP and AL enzymes, can induce gut GABA production and brain GABA/homocarnosine elevation. FOS is a potential therapeutic agent in epilepsy treatment, and a combination with the anti-epileptic drug Vig further strengthened its potency. Increasing gut and brain GABA/homocarnosine levels, increasing oxidative and anti-inflammatory defenses, and improved tight junctions of the brain may be the underlying mechanisms of FOS in ameliorating epilepsy. Finally, homocarnosine may play a role in regulating hyperactive-, depression-, and anxiety-like behaviors. This study provides new insights into development of nutritional approaches for mood, behavior, and neurological disorder management.