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Sedative-Hypnotic and Receptor Binding Studies of Fermented Marine Organisms

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Abstract

This study was performed to investigate the sedative-hypnotic activity of γ-aminobutyric acid (GABA)-enriched fermented marine organisms (FMO), including sea tangle (FST) and oyster (FO) by *Lactobacillus brevis BJ20 (L. brevis BJ20)*. FST and FO were tested for their binding activity of the $GABA_A$-benzodiazepine and $5\text{-}HT_{2C}$ receptors, which are well-known molecular targets for sleep aids. We also measured the sleep latency and sleep duration during pentobarbital-induced sleep in mice after oral administration of FST and FO. In $GABA_A$ and $5\text{-}HT_{2C}$ receptor binding assays, FST displayed an effective concentration-dependent binding affinity to $GABA_A$ receptor, similar to the binding affinity to $5\text{-}HT_{2C}$ receptor. FO exhibited higher affinity to $5\text{-}HT_{2C}$ receptor, compared with the $GABA_A$ receptor. The oral administration of FST and FO produced a dose-dependent decrease in sleep latency and increase in sleep duration in pentobarbital-induced hypnosis. The data demonstrate that FST and FO possess sedative-hypnotic activity possibly by modulating $GABA_A$ and $5\text{-}HT_{2C}$ receptors. We propose that FST and FO might be effective agents for treatment of insomnia.

Key Words: Fermented marine organisms, $GABA_A$ receptor, $5\text{-}HT_{2C}$ receptor, Pentobarbital-induced sleep, Sedative-hypnotic activity

INTRODUCTION

Insomnia is one of most prevalent sleep disorders and approximately more than a third of adults populations suffer from chronic insomnia and poor sleep. These estimates are even higher among adults with coexisting medical or psychiatric illness (Doghramji, 2006). Traditionally, sedative-hypnotic drugs such as γ-aminobutyric acid (GABA)-A benzodiazepine (BZD) receptor or serotonin (5-HT) receptor agents, have been prescribed to treat sleep disturbances, but certain drugs in this class have limited benefits due to side effects such as impairments of cognitive function and memory, changes in appetite, diarrhea, daytime drowsiness, headache or unusual dreamsn (Attele *et al*., 2000; Roth *et al*., 2001; Borja and Daniel, 2006). Therefore, natural sleep aids with sedative-hypnotic effects are being increasingly sought after by the general population as an alternative to prescription drugs to improve sleep quality and avoids side effects. Many studies on the sedative-hypnotic efficacy of terrestrial plants have been performed. Several marine plants among them are known to have biological activities, but the use of these products has been restricted as a potential source of natural sleep drugs.

The molecular targets of medicinal plants producing sedative-hypnotic activity have more recently focused on regulation of GABAergic and serotonergic neurotransmission in the central nervous system (CNS), which is involved in stress and sleep regulation (Attele *et al*., 2000; Abourashed *et al*., 2004). GABA, the major inhibitory neurotransmitter in the CNS, functions to maintain a balance between neuronal excitation and inhibition (Johnston, 2005). GABAergic neurotransmission plays a key role in sleep regulation, and the BZD binding site on the GABA receptor is a target for most sedative-hypnoptic agents (Johnston, 2005). BZD agents potentiate the ability of GABA to cause membrane hyperpolarization by allowing a Cl-influx. As a result, the inhibition of neurotransmission is achieved, and subsequently these agents produce sedative-

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hypnotic, anxiolytic, and anticonvulsant activities (Smith and Simpson, 2003). 5-HT and its receptors have been known to play an important role in control and mechanism of sleep. The 5-HT receptors have been classified as seven groups, 5-HT_{1-7}. The 5-HT_2 class consists of three subtypes, 5-$HT2_{A,B, and C}$ which are structurally associated with the superfamily of G-protein-coupled receptors. In particular, 5-HT_{2C} receptors have been implicated to modulate sleep and wakefulness and to be potential target for sleep-promoting drugs. For example, administration of a selective 5-HT_{2C} receptor antagonist significantly increased slow wave sleep and reduced rapid-eye-movement sleep in the rat (Smith *et al*., 2002). Several studies have shown that majority of the 5-HT2C receptor-immunoreactive cells are colocalized with GABAergic neurons in the brain areas (Liu *et al*., 2007). Thus, these mixed serotonergic and GABAergic mechanisms may be highly implicated in modulating sedative or the anxiolytic-like effects of sleeping-promoting agents.

Marine organisms, including sea tangle and oyster, have long been a part of food resources to promote health in Japan and Korea, and have received increasing attention over the past decade. Marine plants provide essential bioactive compounds, such as carotenoids, dietary fiber, protein, essential fatty acids, vitamins, and minerals (Athukorala *et al*., 2006, Athukorala *et al*., 2007). Many studies have reported that extracts of marine plants possess biological activities. For example, sea tangle (brown algae; *Laminaria japonica*) extracts was reported to have protective effects against oxidative cell damage, antioxidant and immunomodulatory effects (Athukorala *et al*., 2007, Kang *et al*., 2012). In addition, oyster (*Ostrea gigas*) extracts have possessed antimicrobial and antioxidant activity (Fuda *et al*., 2015). In related research, we used L. brevis to develop fermented sea tangle (FST) by isolated from kimchi, and demonstrated enriched GABA contents (5.56% dry weight) and antioxidatant activity (Kang *et al*., 2012). In addition, many studies have reported that GABA-enriched foods including green tea and brown rice produced antihypertensive effects (Aoki *et al*., 2003, Shizuka *et al*, 2004) As a result, GABA-enriched fermented marine organisms are generally believed to be a good candidate for the production of safe biologically-active substances. However, there is still limited information on the pharmacological aspects of fermented marine organisms in the treatment of insomnia. To address this issue, we investigated the sedative-hypnotic effects of GABA-enriched fermented marine organisms on pentobarbital-induced sleep behavior and evaluated its possible mechanism through $GABA_A$ and 5-HT_{2C} receptor binding assays.

MATERIALS AND METHODS

Preparation of fermented marine organisms

Fermented marine organisms were prepared as described by Kang *et al*. (2012). For the production of GABA-enriched preparations, FMO were added to water at a ratio of 1:15 (w/v) followed by 3% yeast extract and 1% glucose, each based on the amount of yeast extract (control), sea tangle and oyster, to aid in the fermentation process. After autoclaving at 121°C for 30 min, each mixture received 5% v/v of a culture of *L. brevis* (Korean Collection for Type Culture accession number 11377BP). The suspension was thoroughly mixed and incubated at 37°C.

The GABA content in the culture broth was measured as follows. A 1 ml sample was taken and diluted 20-fold using 0.02N HCl. The dilution solution (2 ml) was filtered through a 0.2 μm membrane filter (dismic-25cs, Toyo Roshi Kaisha, Tokyo, Japan). The GABA and glutamate in the filtered sample (20 μl) were measured using Amino acid analyser (AAA L-8900, Hitachi High-Technologied Co., Tokyo, Japan) with a flow rate of 0.3 ml/min.

Animals

The male Spraque-Dawley rats, weighting 180-200 g, were used for the receptor binding assay. The male ICR mice were used, weighting 18-22 g each, for the pentobarbital-induced sleep test. All animals were purchased from Orient Animal Corp (Kyungki-do, Republic of Korea), and were group-housed under a reversed 12:12 h light-dark cycle (light on from 08:00-20:00 h). The animals had free access to food and water at a room temperature that was controlled from 20-25°C. All animals were handled daily for at least 7 days prior to the experiment. All animal care and testing conditions were in accordance with the IACUC (Institutional Animal Care and Use Committee) in College of Korean Medicine, the Kyung Hee University.

[^{3}H]-flumazenil binding assay

The $GABA_A$-BZD receptor affinity test was carried out as described by Cho *et al*. (2010). Briefly, the cerebral cortex of each rat was homogenized in 20 volumes of Tris-citrate buffer (50 mM, pH 7.1) and centrifuged at 27,000×g for 15 min. The pellets were washed three times with 20 volumes of Tris-citrate buffer using the same centrifugation conditions. The final washed pellet was resuspended in 20 volumes of Tris-HCl buffer (50 mM, pH 7.4) and incubated at 37°C for 30 min to remove endogenous GABA, followed by centrifugation at 27,000×g for 10 min. The pellet was resuspended in Tris-citrate buffer (500 ml binding buffer per gram of original tissue) and used for the binding assay. Membrane suspension (180 μl) was added to 10 μl of the test solution (0.001-20 mg/ml) and 10 μl of 1 nM (final concentration) of [^{3}H]-flumazenil (Ro 15-1788; Perkin Elmer Life and Analytical Sciences Waltham, MA, USA) in a 96-well plate, mixed, and incubated at 4°C for 40 min. The binding reaction was terminated by filtration onto Whatman GF/C glass fiber filter with ice cold Tris-HCl buffer. The filters were dried for 30 min and suspended in Wallac Microbeta plate scintillation fluid. The amount of radioactivity on the filters was determined using a Wallac 1450 Microbeta liquid scintillation counter (Perkin Elmer Life and Analytical Sciences). Specific binding was calculated as total binding minus non-specific binding, which were determined using binding buffer and clonazepam (1mM, final concentration), respectively.

[^{3}H]-mesulergine binding assay

5-HT_{2C} receptor binding assay was performed expressing human 5-HT_{2C} receptor membrane (Perkin Elmer Life and Analytical Sciences). Membrane diluted in 50 mM Tris-HCl (pH 7, 4 mM $CaCl_2$, 0.1% ascorbic acid) at a concentration of 4 μg/ml. Membrane suspension (180 μl) was added to 10 μl of a test solution (0.001-20 mg/ml) and 10 μl of 1 nM (final concentration) of [^{3}H]-mesulergine (Perkin Elmer Life and Analytical Sciences, Waltham, MA, USA) in 96-well plate, mixed, and incubated at 27°C for 60 min. The binding reaction was termi-

nated by filtration onto Whatman GF/C glass fiber filter with ice cold Tris-HCl buffer (50 mM, pH 7.4). The filters were dried for 30 min and suspended in Wallac Microbeta plate scintillation fluid. The amount of radioactivity on the filters was determined as described above. Specific binding was calculated as described above, except using final concentrations of 100 μM mainserin.

Pentobarbital-induced sleep test in mice

The pentobarbital-induced sleep test was carried out according to the modified method described by Ma *et al*. (2009).

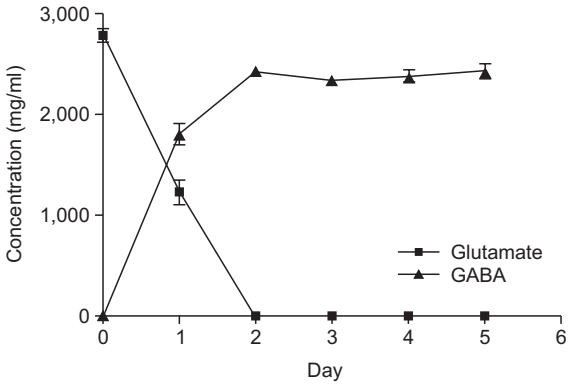

Fig. 1. Changes in GABA and glutamic acid in FO (A) and FST (B) solution during fermentation with *L. brevis*.

All experiments were performed between 1:00 and 5:00 pm and mice were fasted for 24 h prior to the experiment. For oral administration, FO or FST or diazepam (DZP, 2 mg/kg), as positive control were suspended in 0.5% (w/v) carboxymethylcellulose (CMC)-saline. FO or FST (200 and 400 mg/kg) or diazepam (DZP, 2 mg/kg) were administered orally to animals, 30 min before pentobarbital injection. Following the intraperitoneal injection (i.p.) of pentobarbital (hypnotic dosage, 40 mg/kg), each mouse was observed for measurement of sleep latency and sleeping time. The mice lost the righting reflex over 3min were considered to be asleep. The sleep latency was recorded from the pentobarbital injection to the sleep onset and sleeping time was defined as the difference of time between loss and recovery of the righting reflex.

Statistical analyses

In the binding assay, displacement binding curves were filtered to a one-site competition binding model. The displacement (%) of radio-ligand binding was determined as $[1-(DPM_{sample}-DPM_{NSB})/(DPM_{TB}-DPM_{NSB})]\times100$. In the pentobarbital-induced sleep experiments, all data were analyzed using a one-way analysis of variance (ANOVA) for multiple comparisons where necessary as means ± S.E.M. Statistical differences among the groups were further analyzed using the Tukey's post hoc method. The level of significance was set to $p<0.05$.

Table 1. Free amino acid contents of fermented sea tangle solutions with *L. brevis*

Amino acids	Fermented sea tangle solution		Fermented oyster solution	
	With *L. brevis* (0 days later) mg/l sample	With *L. brevis* (5 days later) mg/l sample	With *L. brevis* (0 days later) mg/l sample	With *L. brevis* (5 days later) mg/l sample
Phosphoserine	24.6	33.0	9.88	147.06
Taurine	14.4	26.3	3785.20	3785.93
Phosphoethanolamine	8.09	7.65	5.59	0.00
Aspartic acid	1834	1285	1612.53	2048.35
Threonine	1.43	4.37	14.68	81.14
Serine	32.9	1.67	27.73	181.18
Glutamic acid	2651	0.00	8200.50	0.00
Glycine	7.86	30.2	9.66	688.07
Alanine	198	814	0.00	0.00
DL-2-Aminobutric acid	5.31	33.3	0.00	10.66
Valine	4.79	44.8	12.77	4.21
Phenylalanine	0.00	26.7	5.21	76.07
β-Alanine	2.26	10.1	1.04	133.54
Aminoisobutyric acid	1.15	4.55		
β-Aminobutyric acid (GABA)	0.00	2465	0.00	4845.43
Aminoethanol	14.9	14.2	8.58	15.65
Ornithine	2.84	2.80	4.57	1.38
Lysine	0.00	9.62	5.45	51.96
Histidine	0.00	0.00	0.98	59.38
Arginine	2.40	12.4	6.44	5.28
Proline	116	85.3	88.57	0.00

Table 2. [^{3}H] flumazenil binding of fermentated marine organisms to the $GABA_A$-BZD receptor

Species	Displacement (%) of [^{3}H] flumazenil binding at different plant extract concentration (mg/ml)							
	0.001	0.01	0.1	1	2.5	5	10	20
Control	5.6 ± 1.4	4.1 ± 2.6	1.6 ± 2.2	5.3 ± 0.8	-1.9 ± 4.3	5.0 ± 1.0	2.8 ± 3.9	-2.2 ± 5.0
FO	-12.9 ± 2.2	-11.7 ± 0.9	-12.6 ± 0.5	-10.9 ± 1.4	6.3 ± 2.4	18.4 ± 1.2	31.5 ± 2.3	33.9 ± 0.9
FST	-10.7 ± 3.3	-4.7 ± 2.0	0.3 ± 2.2	4.5 ± 2.3	24.1 ± 1.1	41.3 ± 2.4	45.9 ± 1.8	51.4 ± 3.3

Table 3. [^{3}H]-mesulergine binding of fermentated marine organisms to the 5-HT_{2C} receptor

Group	Displacement (%) of [^{3}H] mesulergine binding at different plant extract concentration (mg/ml)							
	0.001	0.01	0.1	1	2.5	5	10	20
Control	11.8 ± 4.1	0.5 ± 7.4	-1.3 ± 7.1	15.2 ± 6.8	5.3 ± 10.2	17.3 ± 1.2	-6.3 ± 2.5	3.9 ± 2.7
FO	-0.8 ± 7.4	4.2 ± 9.9	15.5 ± 2.8	17.9 ± 3.2	45.1 ± 3.9	58.3 ± 3.4	61.9 ± 0.8	62.2 ± 0.6
FST	20.5 ± 3.9	22.3 ± 1.7	24.4 ± 1.4	29.6 ± 2.5	51.9 ± 4.9	53.0 ± 1.5	58.2 ± 2.1	59.1 ± 1.3

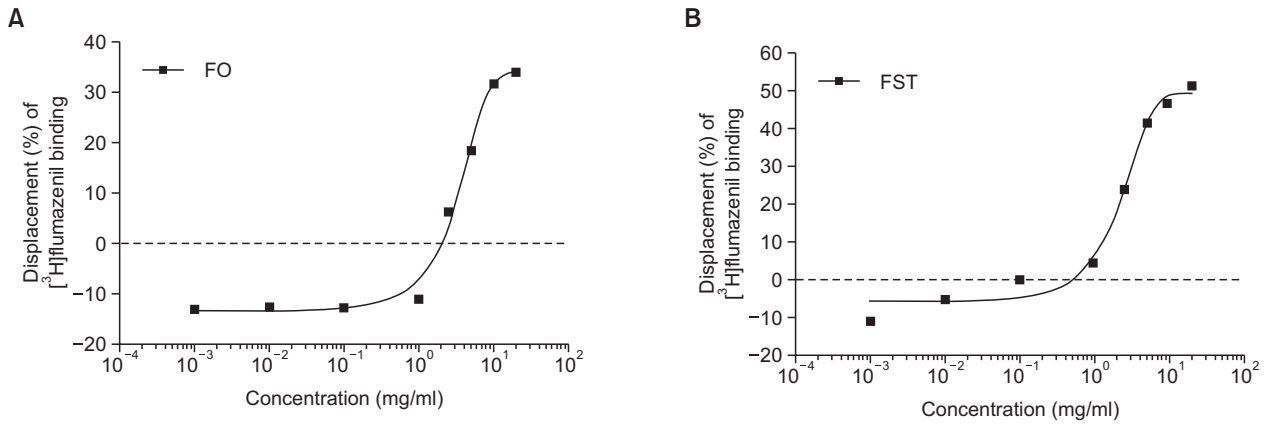

Fig. 2. Dose-response curves and IC_{50} values of FO (A) and FST (B) in the $GABA_A$-BZD receptor binding assay. Each data point is expressed as mean ± SD (n=3).

RESULTS

The changes of GABA and glutamate in fermented marine organisms during fermentation by *L. brevis*

Fig. 1 shows the changes of GABA and glutamic acid in the sea tangle and oyster solution during fermentation with *L. brevis*. The glutamic acid contents in sea tangle and oyster solution were 2,651 and 8,200 mg/L, respectively before fermentation. However, its concentrations were dramatically decreased during fermentation. GABA was not detected in the intact sea tangle and oyster solution. However, GABA dramatically was increased during fermentation with *L. brevis*. The concentrations were 2,465 and 4,845.43 mg/L after 5 days of fermentation, respectively. These results indicate that the glutamic acid in the sea tangle and oyster solution was completely converted to GABA by *L. brevis*.

The free amino acid contents of the fermented sea tangle and oyster solution with *L. brevis* were shown in Table 1. After fermentation of sea tangle, most of the glutamic acid were converted to GABA, and some levels of amino acids such as aspartic acid, serine, and glycine were decreased. The contents of alanine, valine, glycine, phenylalaline and lysine dramatically were increased after fermentation of sea tangle. Furthermore, glutamic acid of oyster has been converted to GABA and phophoserine, aspartic acid, threonine, serine and glycine after fermentation.

Binding affinity of fermented marine organisms to $GABA_A$ and 5-HT_{2C} receptor

Table 2 and 3 presents the % displacement of [^{3}H] flumazenil ($GABA_A$ receptor agonist) and [^{3}H] mesulergine (5-HT_{2C} specific agonist) binding with unlabeled FMO obtained with seven concentrations. FST showed moderately dose-dependent activity to $GABA_A$ receptor, which displaced over 50% of [^{3}H] flumazenil binding at a concentration of 20 mg/ml. In addition, FO had a weak bonding activity for $GABA_A$ receptor at a concentration of 20 mg/ml. The IC_{50} values of FO and FST were 2.25 ± 1.1 and 1.05 ± 1.15 mg/ml, respectively as seen in Fig. 2. In the 5-HT_{2C} receptor binding assay, FO displayed over 60% of [^{3}H] mesulergine binding at a concentration of 20 mg/ml. FST showed moderately dose-dependent activity in 5-HT_{2C} binding assay similar to its affinity of $GABA_A$ recep-

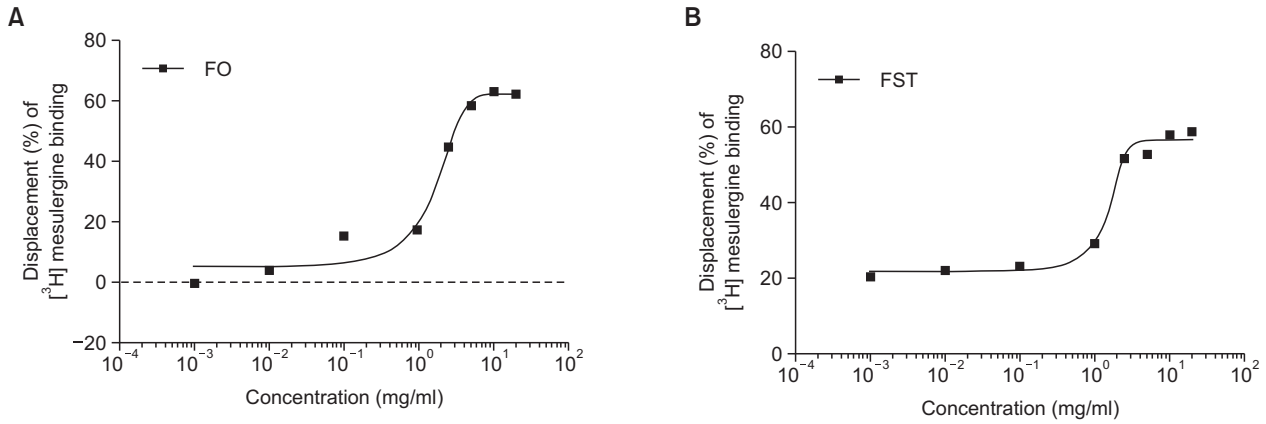

Fig. 3. Dose-response curves and IC_{50} values of FO (A) and FST (B) in the 5-HT_{2C} receptor binding assay. Each data point is expressed as mean ± SD (n=3).

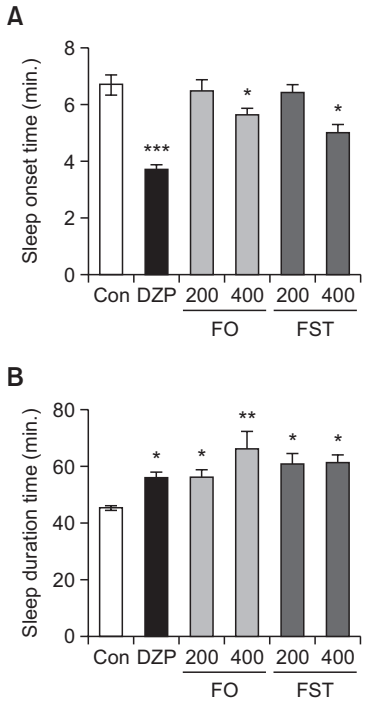

Fig. 4. Effects of FMO on sleep latency (A) and sleep duration (B) in mice induced by hypnotic dose (40 mg/kg, i.p.) of pentobarbital. Mice received pentobarbital 30 min after administration of FMO. CON: control (saline 10 ml/kg, p.o.) Each column represents the mean ± SEM. **p*<0.05, ***p*<0.01, ****p*<0.001, significant as compared to the control group.

tor, which displaced over 50% of [^{3}H] mesulergine binding at a concentration of 20 mg/ml. The IC_{50} values for FO and FST were determined to be 1.34 ± 1.11 and 1.56 ± 0.26, respectively as seen in Fig. 3.

Effects of fermented marine organisms on pentobarbital-induced sleep in mice

The sedative-hypnotic effects of FMO were tested by measuring the sleep onset and sleep duration time pentobarbital-induced sleep after oral administration of FMO in mice. ANOVA analysis of pentobarbital test revealed that sleep onset and sleep duration time were significantly different across all groups ($F_{5,59}$=15.394, p<0.001; $F_{5,59}$=5.459, p<0.01, respectively). The oral administration of FST and FO produced a dose-dependent decrease in sleep latency and increase in sleep duration in mice with hypnotic dose (40 mg/kg) of pentobarbital as seen in Fig. 4. The most pronounced hypnotic activity of FO was observed at 400 mg/kg in the sleep onset time (p<0.05) (Fig. 4A) and sleep duration time (p<0.01) (Fig. 4B), compared to the control group. FST significantly decreased sleep onset time (p<0.05) (Fig. 4A) and increased sleep duration time (p<0.05) (Fig. 4B) at a dose of 400 mg/ml compared with the control mice. However, FST and FO at a low dose (200 mg/kg, p.o) had no significantl hypnotic effects on the sleep onset time. As a positive control, DZP (2 mg/kg, p.o) significantly potentiated the pentobarbital-induced sleep behaviors, compared with control group (p<0.001 for sleep onset time, p<0.05 for sleep duration time).

DISCUSSION

The results of the current study demonstrate the sedative-hypnotic activity of gamma-amino butyric acid (GABA)-enriched fermented marine organisms (FMO), including sea tangle (FST) and oyster (FO) by *Lactobacillus brevis BJ20* (*L. brevis*). It was shown that glutamic acid of marine organisms was converted to GABA after fermentation by *L. brevis*. In $GABA_A$ and 5-HT_{2C} receptor binding assays, FST displayed an effective concentration-dependent binding affinity to $GABA_A$ receptor, similar to the binding affinity to 5-HT_{2C} receptor. FO exhibited higher affinity to 5-HT_{2C} receptor, compared with the $GABA_A$ receptor. The oral administration of FST and FO produced a dose-dependent decrease in sleep latency and increase in sleep duration in pentobarbital-induced hypnosis. Our data suggest that FST and FO possess sedative-hypnotic activity possibly by modulating $GABA_A$ and 5-HT_{2C} receptors.

In the 5-HT_{2C} receptor biding assay, FO showed a moderate concentration-dependent binding activity with an IC_{50} value of 1.34 mg/ml, whereas FO had a weak binding affinity to $GABA_A$-BZD receptor with an IC_{50} value of 2.25 mg/ml. In addition, FST had similar binding activity to $GABA_A$-BZD and 5-HT_{2C} re-

ceptor with an IC_{50} value of 1.05 and 1.56 mg/ml, respectively. This result indicates that FMO contains at least two natural ligands which bind to the $GABA_A$-BZD and 5-HT_{2C} receptors and is more sensitive to $GABA_A$ receptor than 5-HT_{2C} receptor. The results are in agreement with the reports that FMO contains active compounds such as GABA sedative-hypnotic effects mediated by potentiation of GABA receptors (Johnston, 2005).

Although the results of analytical and binding assay indicated the possibility that fermented marine organisms including FST and FO contain natural ligands that bind to the $GABA_A$-BZD and 5-HT_{2C} receptors, it is difficult to ascertain blood-brain barrier (BBB) penetration of these FMO. Thus, we confirmed the sedative-hypnotic effects of fermented marine organisms through a pentobarbital-induced sleep in mice. The pentobarbital-induced sleep test can be a useful tool for examining the inhibitory effects on the CNS, especially for investigating influences on GABAergic system (Ma *et al*., 2009, Cho *et al*., 2010). It has been demonstrated that many hypnotic and anti-anxiety drugs prolong the pentobarbital-induced sleep time (Silva *et al*., 2007, Ma *et al*., 2009).

GABA-enriched foods have recently been implicated as effective and safe bioactive substances. Several studies have reported that marine organisms are useful as a functional food source that includes essential bioactive compounds, such as protein, essential fatty acids, vitamins and minerals, neuroactive compounds of catecholamine (Athukorala *et al*., 2006, Lee *et al*, 2010). Ginseng or ginseng or ginsenosides isolated from ginseng extract have shown to have high affinity to $GABA_A$ (Kimura *et al*, 1994) and 5-HT_{2C} receptors (Cho *et al*., 2010) and also have reported to increase non-rapid eye movement sleep (Lee *et al*, 2012). Therefore, it has been suggested that GABA-enriched foods may have beneficial effects on sleep behaviors. We tested this hypothesis and we found that administration of FST and FO produced a dose-dependent decrease in sleep latency and increase in sleep duration with hypnotic dose of pentobarbital. FO and FST produced a marked hypnotic activity at 400 mg/kg in the sleep onset time and sleep duration time, compared to the control group. The pentobarbital-induced sleep behaviors were significantly potentiated by DZP (2 mg/kg, p.o), which is commonly prescribed to treat psychiatric diseases including anxiety and insomnia. DZP increase action of GABA by binding to the benzodiazepine site on the $GABA_A$ receptor, producing behavioral effects. In the present study, marine organisms are more effective in potentiating sleep behaviors than a positive control DZP. These differential effects may be explained by the fact that DZP only increases effects of GABA by binding to the benzodiazepine site on the $GABA_A$ receptor, whereas FST and FO bind to both $GABA_A$-BZD and 5-HT_{2C} receptors, and stimulate synergistically 5-HT and GABAergic systems in the brain.

The present study demonstrated that FST and FO have the hypnotic-sedative effects on sleep-related behaviors, showing that fermented marine organisms lead to decrease sleep latency and increase sleep duration. These results suggest potency of fermented marine organisms as a sleep aid and prove that these effects are closely associated GABAergic and serotonergic systems and may be mediated through these transmitter systems. It is certain that these mixed serotonergic and GABAergic mechanisms may be highly implicated in modulating sedative effects of FMO. This suggestion is strengthen by the fact that most of the 5-HT2C receptor-immunoreactive cells are localized in GABAergic neurons in the brain areas (Liu *et al*., 2007). Thus it is possible that FMO activates the 5-HT2C receptors expressed by GABAergic cells in sleep-related brain areas and may produce decrease in sleep latency and increase in sleep duration.

In summary, our results clearly revealed that most of glutamic acid in sea tangle and oyster solution were converted to GABA during fermentation with *L. brevis*. The results of the current experiments indicate that GABA-enriched FO and FST display high affinity to $GABA_A$ and 5-HT_{2C} receptors, and produce hypnotic-sedative activity in pentobarbital-treated mice. These results suggest that FO and FST are effective in improving sleep and that serotonergic and GABAergic mechanisms may be highly implicated in modulating sedative effects of FMO. Further investigation is needed of other derivatives and isolation of the active compounds of fermented marine organisms that cause the hypnotic-sedative activity including their pharmacological mechanisms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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