*Short Communication***Involvement of the Long-Chain Fatty Acid Receptor GPR40 in Depression-Related Behavior**Takashi Nishinaka¹, Takuya Yamashita², Kazuo Nakamoto¹, Fumiyo Kasuya², and Shogo Tokuyama^{1,*}¹Department of Clinical Pharmacy, ²Biochemical Toxicology Laboratory, School of Pharmaceutical Sciences, Kobe Gakuin University, 1-1-3 Minatojima, Chuo-ku, Kobe 650-8586, Japan

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Abstract. The functional role of brain G protein–coupled receptor 40 (GPR40) remains unclear. We investigated GPR40 signaling in depression-related behavior in mice via the forced swim test. A repeated but not a single intracerebroventricular administration of the GPR40 agonist, GW9508, reduced the duration of immobility behavior. Moreover, the levels of hippocampal non-esterified docosahexaenoic acid and arachidonic acid were decreased immediately after the forced swim-ming. These results suggested that brain GPR40 signaling may regulate depression-related behavior.

Keywords: G protein–coupled receptor 40, depression, free fatty acid

Docosahexaenoic acid (DHA) and arachidonic acid (AA) are essential fatty acids found mainly in the membrane phospholipid layer in the mammalian brain (1). These fatty acids are critical for the development and function of the central nervous system (CNS). A recent growing body of evidence has demonstrated that increasing DHA in the brain may have a beneficial effect on psychiatric disorders, such as depression and schizophrenia (2).

G protein–coupled receptor 40 (GPR40) (also known as free fatty acid receptor 1) is activated by long-chain fatty acids such as DHA and AA (3, 4). GPR40 protein is expressed in pancreatic β cells and in the brain (3). In the peripheral system, GPR40 regulates blood glucose levels by increasing glucose-stimulated secretion of insulin (4). The functional role of GPR40 in the CNS remains largely unknown. However, our group has previously demonstrated that DHA-induced antinociceptive effect on acute noxious stimulus may be mediated (at least in part) through GPR40 signaling in the brain (5). Boneva et al. have reported that GPR40 signaling is involved in hippocampal neurogenesis though the signaling pathway, which is identical with brain-derived neurotrophic factor (BDNF) (6). Increasing evidence

has suggested a link between the BDNF system and adult depression, supporting a role in the pathophysiology of the disease. A single administration of BDNF into the dentate gyrus of the hippocampus produces an antidepressant effect (7). The mRNA levels of BDNF and tropomyosin receptor kinase B (TrkB) were decreased in postmortem brain of suicide subjects in the hippocampus (8). Therefore, we hypothesized that hippocampal GPR40 signaling is involved in the pathophysiology of depression.

The aim of this study was to investigate the involvement of brain GPR40 signaling on depression-related behavior in mice using the forced swim test. Male ddY mice (5-week-old) were obtained from SLC (Hamamatsu). Mice were housed 3 – 6 per cage at 23°C – 24°C with a 12-h light/dark cycle. Food and water were available ad libitum. The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals adopted by The Japanese Pharmacological Society. All experiments were approved by the Ethical Committee for Animal Experimentation of Kobe Gakuin University (Kobe) (approval number A120507-31). Mice were forced to swim in a water-filled cylinder [25 cm height $\times$ 15 cm diameter, filled with water (23°C $\pm$ 1°C), 15-cm depth] for 6 min. The duration of immobility was scored during 6 min. Immobility was regarded as no activity except for movement to keep the head above the water. Mice were placed in the open

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field (30 cm × 30 cm²) surrounded by 42-cm-high wall for 5 min. The floor of the open field was divided into 28 identical squares for measuring locomotor activity. The movement of mice was measured via a camera mounted above the open field. The number migrated between the squares was scored. The selective GPR40-agonist GW9508 (Cayman Chemical Co., Ann Arbor, MI, USA) was dissolved in 4% dimethyl sulfoxide (Sigma-Aldrich Japan K.K., Ishikari). In a single administration study, GW9508 (1.0, 10, or 25 µg/mouse) was injected intracerebroventricularly (i.c.v.) 15 min before the forced swim test. In a repeated administration study, GW9508 (1.0 µg/mouse) was injected (i.c.v.) once a day for 3 or 5 days, and the forced swim test was performed 15 min after the final injection. The i.c.v. administration was performed by using a Hamilton microsyringe fitted with a 27-gauge i.c.v. needle. The injected site was both 2-mm caudal and lateral to the bregma and 3 mm in depth from the skull surface. Injection volumes were 5 µL introduced over 5 s. Western immunoblotting was performed, as previously described but with some modifications (5). Hippocampal tissue was homogenized in homogenization buffer. Protein samples (20 µg/lane) were resolved by 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Membranes were incubated overnight at 4°C with the primary antibody, polyclonal anti-GPR40 (1:500) (Abcam, Cambridge, MA, USA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:20,000) (Chemicon International, Temecula, CA, USA). After washing, the membranes were incubated with the secondary antibodies: horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (1:1000) (KPL, Gaithersburg, MD, USA) for GPR40 and anti-mouse IgG (1:10000) (KPL) for GAPDH for 1 h at room temperature. Immunoreactive bands were detected by an enhanced chemiluminescence western blotting analysis system (GE Healthcare, Buckinghamshire, UK) and visualized using a light-capture system (AE-6981) (ATTO Corp., Tokyo). Nonesterified fatty acid comparative analysis was measured, as previously described but with some modifications (9). Mice were sacrificed immediately after forced swimming or without forced swimming. Hippocampal tissue was measured (wet weight) and then homogenized in methanol/acetone (1:1 v/v). The homogenate was centrifuged (16,000 × *g* for 5 min at 4°C). The composition of nonesterified fatty acid was analyzed by an UHPLC-MS/MS system [ultra high performance liquid chromatography (Nexera); MS:LCMS-8030 triple quad 5500 mass spectrometry (Shimadzu Co., Kyoto)] and controlled by the LabSolutions LCMS (version 5.4) (Shimadzu Co.). All data are expressed

as the mean ± S.E.M. Significant differences were evaluated by one-way analysis of variance followed by the Scheffe's multiple-comparison test for comparisons between more than 3 groups or by Student's *t*-test for comparison between 2 groups. Significance was reached at values of $P < 0.05$.

A single administration of GW9508 did not affect the immobility time in the forced swim test [$F(3,19) = 0.6196$, $P > 0.05$] (Fig. 1A). However, the repeated administration of GW9508 for 5 days, but not 3 days, significantly reduced the immobility time compared with the vehicle group (3 days, $P > 0.05$, 5 days, $P < 0.05$) (Fig. 1: B and C). Both single and repeated administration of GW9508 did not affect the locomotor activity (single, $P > 0.05$, repeated, $P > 0.05$) (Fig. 1D). The levels of nonesterified DHA and AA in the hippocampus were significantly decreased immediately after mice were forced to swim for 6 min compared with the control group [(HA, $P < 0.01$; AA, $P < 0.01$) (Fig. 2: A and B). In contrast, the level of expression of GPR40 did not change after the forced swimming ($P > 0.05$) (Fig. 3).

DHA and AA are the major phospholipids of the cell membrane in the brain (1), and are essential for brain development, structure, and function (10). The hippocampus is involved in memory formation, as well as mood control. DHA affects hippocampal neuronal development and synaptic function (11). Furthermore, a change in the levels of polyunsaturated fatty acid in the membrane phospholipids has been observed in the hippocampus of patients with depression (12).

In the present study, we demonstrated that only repeated administration of GW9508 reduced the duration of immobility behavior and the content of non-esterified DHA and AA but not the expression of GPR40 in the hippocampus. In a previous clinical study using positron emission tomography, the daily consumption of AA and DHA was found to be 17.8 and 4.6 mg / 1500 g per brain/day, respectively (13). These two fatty acids are regularly used in the healthy brain, but under pathological conditions their levels are imbalanced. Therefore, maintaining balanced levels of these fatty acids occurs through daily meals. In the present study, decreased hippocampal non-esterified DHA or AA may have been an alternative response to the excess stress of the forced swimming. Our findings suggested that decreased GPR40 signaling contributed to depression-related behavior during the forced swimming. Although the immobility observed in the forced swim test is hard to interpret, its behavior is thought to reflect the state of behavioral despair (14). However, we do not understand why GPR40 protein expression had not changed after the forced swimming test at this stage.

At least three allosterically-linked binding sites on

Fig. 1. Effect of i.c.v. administration of GW9508 on the immobility time in the forced swim test. A) GW9508 (1.0, 10, or 25 $\mu\text{g}/\text{mouse}$) administered 15 min before the forced swim test [vehicle group: $n = 6$, GW9508 (1.0 $\mu\text{g}/\text{mouse}$) group: $n = 6$, GW9508 (10 $\mu\text{g}/\text{mouse}$) group: $n = 4$, GW9508 (25 $\mu\text{g}/\text{mouse}$) group: $n = 7$]. GW9508 (1.0 $\mu\text{g}/\text{mouse}$) was administered once a day for 3 (B) or 5 days (C) and then the forced swim test performed 15 min after the final injection [B) vehicle group: $n = 6$, GW9508 (3 days) group: $n = 6$; C) vehicle group: $n = 11$, GW9508 (5 days) group: $n = 12$]. The results show the immobility time during 6 min. D) Locomotor activity measured 15 min after a single or repeated (5 days) administration of GW9508 (1.0 $\mu\text{g}/\text{mouse}$) (single vehicle group: $n = 5$, single GW9508 group: $n = 5$, repeated vehicle group: $n = 5$, repeated GW9508 group: $n = 5$). * $P < 0.05$ vs. vehicle.

Fig. 2. Changes in the levels of non-esterified fatty acids, docosahexaenoic acid (DHA) and arachidonic acid (AA), in the hippocampus. A) DHA or B) AA in the hippocampus immediately after the forced swimming. Control mice were not exposed to forced swimming. Results are expressed as a percentage of Control. Control group: $n = 5$, forced swimming group: $n = 6$. ** $P < 0.01$ vs. Control.

Fig. 3. Changes in the level of expression of hippocampal GPR40. GPR40 in the hippocampus immediately after the forced swim. The lower panel shows a representative western immunoblot of GPR40 (26 kDa) and GAPDH (37 kDa). Relative levels of GPR40 were normalized to GAPDH. The results are expressed as a percentage of Control. Control group: $n = 5$ and forced swimming group: $n = 6$.

GPR40 have been recently reported (15). The GPR40 agonists, TAK-875 and GW9508, are partial agonists and allosteric modulators that improve the potency of a GPR40 endogenous agonist such as a long-chain fatty acid (16). Therefore, we speculate that the single administration of GW9508 did not reduce the duration of immobility behavior because the endogenous agonist for GPR40 in the hippocampus was reduced immediately after the forced swim test.

In conclusion, repeated administration of GW9508 reduced the duration of immobility behavior. Furthermore, GPR40 signaling in the hippocampus is decreased in the forced swimming. Overall, these findings suggest that GPR40 signaling in the hippocampus contributes to the regulation of depression-related behavior.

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