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Ghrelin improves cognitive function in mice by increasing the production of insulin-like growth factor-I in the hippocampus

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ABSTRACT

The gut hormone and neuropeptide ghrelin improves cognitive function by enhancing synaptic plasticity within the hippocampal formation. However, the underlying molecular mechanisms are not fully understood. We demonstrated that sensory neurons play a critical role in the increase of hippocampal production of insulin-like growth factor-I (IGF-I) by releasing calcitonin gene-related peptide (CGRP), thereby contributing to the improvement of cognitive function in mice. Ghrelin stimulates the sensory circumventricular organs (CVOs) where CGRP is abundantly found. Since nervous connection to the hippocampus is demonstrated in CVOs, it is possible that ghrelin improves cognitive function by increasing the hippocampal production of IGF-I via stimulation of CVOs in mice. We examined this possibility using wild-type (WT) and CGRP-knockout (CGRP-/-) mice. Hippocampal tissue levels of CGRP, IGF-I and IGF-I mRNA were increased, and angiogenesis and neurogenesis were promoted in the dentate gyrus of the hippocampus after intraperitoneal injection of ghrelin in WT mice. Increase of the number of IGF-I immunoreactivity was co-localized with immunoreactivity of the astrocyte marker GFAP (glial fibrillary acidic protein) in the hippocampus, and expression of *c-fos* was increased in CVOs (the area postrema and solitary tract nucleus), parabrachial nuclei, and the hippocampus in WT mice administered ghrelin. Ghrelin administration improved spatial learning in the Morris water maze in WT mice. None of effects of ghrelin in WT mice were observed following administration of ghrelin in CGRP-knockout (CGRP-/-) mice. These observations suggest that ghrelin may increase the hippocampal production of IGF-I via increase of CGRP release from neurons in CVOs,

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thereby improving cognitive function in mice.

Key words: IGF-I, insulin-like growth factor-I; CGRP, calcitonin gene-related peptide; CVOs, circumventricular organs.

INTRODUCTION

The 28-amino acid motilin-related peptide ghrelin is originally purified from the rat stomach and is known as an endogenous ligand of the growth hormone secretagogue receptor 1a (GHSR-1a)[1]. Ghrelin is capable of modulating the cell proliferation and survival[2-4]and has several biological effects such as regulation of feeding, gastric secretion and motility, and fat mass deposition[5-8]. In addition to these biological activities, ghrelin has been shown to improve spatial learning by acting on hippocampal neurons and induce formation of new synapse in the CA1 region[9], which is consistent with the idea that ghrelin is involved in the appetitive phase of ingestive behavior when it is important to find out food in the environment by recalling of stored representations of prior experience with food[10]. However, since ghrelin is shown to unable to cross the blood-brain barrier[11], mechanisms by which ghrelin improves cognitive function by increasing the neuronal plasticity in the hippocampus are not fully understood.

Insulin-like growth factor-I (IGF-I) is a basic peptide composed of 70 amino acids, with ubiquitous distribution in various tissues and cells[12]. It mediates the growth-promoting actions of growth hormone and plays an important role in postnatal and adolescent growth[12]. IGF-I has been shown to enhance excitatory synaptic transmission in the CA1 region of the hippocampus and to improve spatial learning by inducing neurogenesis in the hippocampus[13]. The impaired spatial learning in mice with low serum levels of IGF-I is reversed by exogenous administration of IGF-I[14]. A close correlation has been shown between the plasma IGF-I levels and cognitive function in older individuals[15]. These observations strongly suggest that IGF-I may improve cognitive function by increasing the plasticity and promoting neurogenesis in the hippocampus.

It is reported that sensory neurons play a critical role in the increase of hippocampal production of IGF-I by releasing calcitonin gene-related peptide (CGRP) in mice[16]. CGRP, a 37-amino acid neuropeptide, is produced by the alternative splicing of the calcitonin gene[17]. It is widely distributed in the central and peripheral nervous systems[18].

GHSR-1a is widely expressed in peripheral and central tissues, including the hypothalamus, hippocampus, and cortex[19]. Main sites of ghrelin action in the hindbrain are the dorsal vagal complex, an autonomic center that includes the nucleus tractus solitarius (NTS), the dorsal

motor nucleus of the vagus nerve, and the area postrema (AP)[20]. Since NTS and AP are included in the specialized group of CNS structure called the sensory circumventricular organs (CVOs) that lack the blood-brain barrier, these areas easily detect circulating hormones that are unable to cross the blood-brain barrier without a transport system[21, 22]. CGRP-containing fibers are abundantly found in AP and NTS[23], and nonprincipal neurons containing CGRP in the hippocampus are projected from NTS via the parabrachial nuclei (PBN)[16].

Taken together, these observations raise the possibility that circulating ghrelin increases the hippocampal production of IGF-I by increasing CGRP release from neurons in AP, NTS, PBN, and the hippocampus, thereby improving cognitive function.

In the present study, we examined whether administration of ghrelin improves cognitive function by increasing the hippocampal IGF-I production via stimulation of AP, NTS, and PBN in wild-type (WT) and CGRP-knockout (CGRP-/-) mice.

MATERIALS AND METHODS

Animal Model

Age-matched (10 to 12-wk-old, 21 to 24 g) male C57BL/6wild-type (WT) (Nihon SLC, Hamamatsu, Japan) and αCGRP-deficient (CGRP-/-) mice were used in each experiment. The generation of CGRP-/- mice were described previously[24]. They were maintained under standard conditions of temperature (23-25℃) and on a 12 h light/dark cycle. Food and water were provided *ad libitum*. The care and handling of the animals were in accordance with the National Institute of Health guidelines. All the experimental procedures described below were approved by the Nagoya City University Animal Care and Use Committee. Mice were treated by intraperitoneal injection of 100 μl of 0. 1 μg/μl ghrelin solution dissolved in PBS or with PBS alone. Injections took place at 9 a.m. and 3 p.m. on four consecutive days as described previously[9]. The animals were sacrificed between 30 min and 1 h after the last injection on the fourth day besides the animals used for behavioral test and immunohistochemistry for CD31, GFAP or Doublecortin with BrdU. Mice were intraperitoneally injected with (BrdU) (50 mg/kg; Sigma Chemical Co.) for 5 consecutive days. Those animals were sacrificed between 30 min and 1 h after the final injection.

Measurement of hippocampal CGRP level

Tissue levels of CGRP were determined in mice by a modification of the methods described previously[25]. Hippocampus were weighted and homogenized in 0. 5 ml of 2N acetic acid. Homogenates were bathed in 90℃ water for 20 min and then centrifuged at 4500 g for 10 min (4℃). CGRP was extracted from the supernatant using reverse-phage C18 columns (Amersham, Little Chalfont, UK). Columns were prepared by washing with 5 ml methanol

onto the column followed by a wash with 20 ml of 0.1% trifluoroacetic acid, and the solvent was evaporated under a stream of nitrogen gas. The concentration of CGRP was assayed by using a specific enzyme immunoassay kit (SPI-BIO, Massy, France). The sensitivity of the CGRP assay was 10 pg/ml. The antiserum cross-reacted 100% of rodent α- and β- CGRP according to the manufacturer's data sheet. Results are expressed as micrograms of CGRP per gram of tissue.

Measurement of hippocampal IGF-I level

Tissue levels of IGF-I were determined in animals by modification of the methods as described previously[26]. The hippocampus was minced and homogenized in a polytron type homogenizer (2 times of 15s) using 1 ml of 1N acetic acid according to the manufacture's instruction. The homogenates was than centrifuged at 4500 g for 10 min. The supernatants were kept in a deep freezer at −80℃. The concentration of IGF-I was assayed by using a specific enzyme immunoassay kit (Diagnostic Systems Laboratories Inc., Webster, TX).

Quantitative mRNA analysis

Quantitative mRNA analysis was performed as previously described[26]. The tissue was weighed and immersed in liquid nitrogen. Total RNA was extracted from the hippocampus with TRIZOL Reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. RNA extracted was used as a template for cDNA reverse transcription. Sample cDNAs were amplified in the Model 7700 Sequence detector (Applied Biosystems, Perkin Elmer Japan, Chiba, Japan) with primers, dual-labeled fluogenic probes, and a Taqman PCR Reagent Kit (Applied Biosystems, Branchburgh, NJ). Thermal cycler conditions were 10 min at 95℃ for deactivation preceding 40 cycles for 15 sec at 95℃ for denaturation and 1 min at 60℃ for both annealing and extension. Known concentrations of serially diluted IGF-I and β-actin cDNA generated by PCR were used as standards for quantitation of sample cDNA. Copy numbers of cDNA for IGF-I was standardized those for β-actin from same sample.

Immunohistochemistry

For histochemical analysis, mice were anesthetized and perfused with 0.01 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde in PBS. Brains were removed and post-fixed for 24 h in 4% paraformaldehyde in 0.1 M PBS (pH 7.4), and then stored in PBS with 20% sucrose for 24 h. The isolated brains were frozen in an OCT compound (Tissue Tec; Miles, Elkhart, IN, USA) and serial sections at 30 μm thickness were used for immunofluorescence as described[27]. Samples for BrdU staining were treated for DNA denaturation with 2N HCl. Sections were incubated with primary antibodies at the fol-

lowing concentrations: rabbit anti-c-fos polyclonal antibody (1: 500; Santa Cruz Biotechnology Inc., Santa Cruz, CA), mouse anti-IGF-I monoclonal antibody (1: 200; Upstate Biotechnology), rabbit anti-GFAP polyclonal antibody (1: 1000; Dako, Glostrup, Denmark), mouse anti-BrdU monoclonal antibody (1: 100; Invitrogen), rat anti-BrdU monoclonal antibody (1: 400; Abcam), rat anti-CD31 monoclonal antibody (1: 200; BD Biosciences PharMingen, San Diego, CA), and goat anti-doublecortin (DCX) monoclonal antibody (1: 1000; Santa Cruz Biotechnology Inc., Santa Cruz, CA). After incubating at 4℃ overnight and washing, the sections were treated with secondary antibodies, Alexa 488 anti-rabbit IgG for *c-fos*, Alexa Fluor 488 anti-mouse IgG for IGF-I, Alexa Fluor 594 anti-rat IgG for CD31, Alexa Fluor 488 anti-mouse IgG for BrdU (anti-mouse), Alexa Fluor 568 anti-rabbit IgG for GFAP, Alexa Fluor 594 anti-rat IgG for BrdU (anti-rat) (1: 500; Invitrogen), and Alexa Fluor 488 anti-goat for DCX 1 h at room temperature. Negative controls were performed by omitting the primary antibodies during the immunostaining. Samples were then mounted and photographed under light Fluorescence microscope (Axio Image A1, Carl Zeiss)

Morris water maze task

Behavioral testing was conducted as described previously[28]. We used a circular pool (150cm diameter). The pool was filled with water at 30℃ and contained a round-shaped transparent acrylic platform (12 cm diameter). In the task, the platform was submerged 1 cm below the surface of the water and located in the southeast quadrant of the pool throughout the trials. After a mouse was put into the pool, each had a maximum of 90 sec to locate and climb onto the platform (one trial). When a mouse located the platform, it was allowed to stay on it for 20 sec. Mouse that did not find the platform in the allowed time was placed on it by the experimenter and left there for 20 sec. Latency to reach the platform was monitored. Each mouse was subjected to one trial per day. The task consisted of 5 days of trials. Two hours after the last trial, the probe test was carried out. For this test, the platform was removed from the pool and the trial was performed with the cutoff time of 90 sec. The time spent in the target area (zone radius: 30 cm, three times the target diameter) was recorded as a percentage of the trial time in the pool.

Quantification and stastistical analysis

The BrdU-positive cells in the subgranular (SGZ), granule cell layer (GCL) and in the hilus of the hippocampus was counted using the stereological counting method. Five sections from the hippocampus were cut beginning 1. 58 mm caudal and 1. 94 mm anterior to the bregma with intervals of 100 μm. To assess the phenotype of BrdU-positive cell in double-immunofluorescence, a mean value for each marker was obtained from 5 sections from 5

mice. The number of c-fos labeled cells was quantified in the area postrema (AP), parabrachialnuclei (PBN), the solitary tract nucleus (NTS), and the hilus of the hippocampus. The number of cells from AP, NTS, PBN and hilus was counted from three sections of each brain (n = 3 for each group) corresponding to planes 7.32, 6.64, 4.96 and 1.86 mm anterior to bregma.

Data are expressed as the mean ± SD. The results were compared using an ANOVA followed by Dunnett's multiple comparison test or the unpaired student's *t*-test (Fig. 3). A level of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of ghrelin on tissue levels of CGRP, IGF-I and IGF-I mRNA in the hippocampus of WT and CGRP-/- mice

To examine whether intraperitoneal administration of ghrelin for 4 consecutive days increases the hippocampal production of IGF-I by promoting CGRP release from neurons in mice, we determined tissue levels of CGRP, IGF-I and IGF-I mRNA in the hippocampus after administration of ghrelin in WT and CGRP-/- mice. At baseline, tissue levels of CGRP, IGF-I and IGF-I mRNA in the hippocampus of WT mice were significantly higher than those in the hippocampus of CGRP-/- mice (Fig. 1). Administration of ghrelin significantly increased hippocampal tissue levels of CGRP, IGF-I and IGF-I mRNA in WT mice ($p < 0.01$), whereas such increases were not observed in the hippocampus of CGRP-/- mice (Fig. 1).

Effect of ghrelin on immunohistochemical expression of IGF-I in the hippocampus of WT mice

Immunohistochemical expression of IGF-I was increased in the hippocampus of WT mice after administration of ghrelin (Fig. 2). Double-immunofluorescence staining for IGF-I and the astrocyte marker GFAP showed that immunoreactivity of IGF-I was co-localized with that of GFAP (Fig. 2). Increase in the number of immunoreactivity of IGF-I co-localized with that of GFAP was observed in the hilus of the dentate gyrus (DG) after administration of ghrelin (Fig. 3).

Effect of ghrelin on hippocampal neurogenesis and angiogenesis in WT and CGRP-/- mice

The number of BrdU-immunoreactive cells in DG was significantly higher in WT mice than in CGRP-/- mice (Fig. 4A). The number of BrdU-immunoreactive cells was significantly increased in DG of WT mice after administration of ghrelin ($p < 0.01$), whereas no such increase was observed in CGRP-/- mice. Co-localization of BrdU immunoreactivity with immunoreactivity for the neurogenesis marker doublecortin (DCX), the vascular endothelial cell marker CD31, and the astrocyte marker GFAP was examined to determine the pheno-

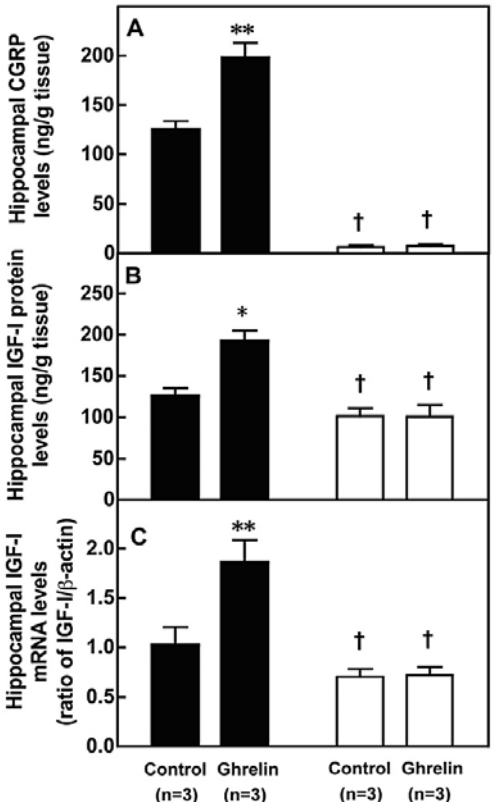

Fig. 1. Effect of ghrelin on tissue levels of CGRP (A), IGF-I (B) and IGF-I mRNA (C) in the hippocampus of WT and CGRP-/- mice

Hippocampi were removed after intraperitoneally injection of Ghrelin (10μg) twice per day for four consecutive days. Each bar represents mean ± S. D. derived from three animal experiments. Solid bars, WT mice; open bars, CGRP -/-mice. *, $p < 0.05$ versus control, **, $p < 0.01$ versus control; †, $p < 0.01$ versus WT mice.

type of progenitor cell progeny in DG after ghrelin administration in WT and CGRP-/- mice (Fig. 4). Significantly higher number of BrdU+/CD31 + cells, BrdU+/DCX+ cells, and BrdU +/GFAP+ cells was observed in DG of WT mice than in that of CGRP-/- mice (Fig. 4). Significant increase of the number of BrdU+/CD31 + cells and BrdU+/DCX+ cells, but not BrdU +/GFAP+ cells, in DG was observed after administration of ghrelin in WT mice (Fig. 4).

Effect of ghrelin on c-fos expression in the brain nuclei of WT and CGRP-/- mice

The hippocampus has been shown to receive signal input from the parabrachial nuclei

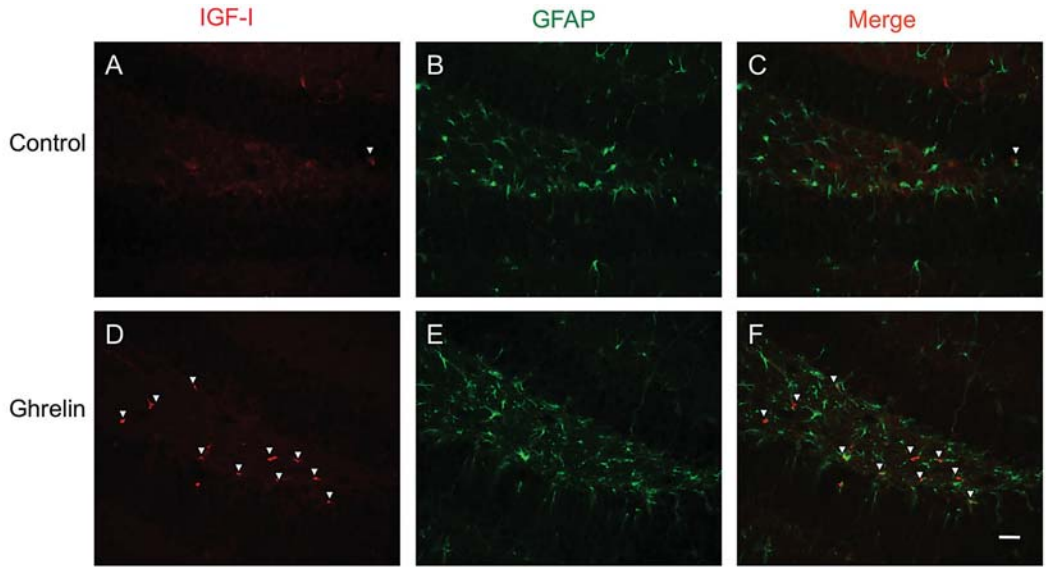

Fig. 2. Effect of ghrelin on immunohistochemical expression of IGF-I in the hippocampus of WT mice
Immunohistochemical expression of IGF-I in the dentate gyrus (DG) of WT mice was increased by ghrelin treatment (D) compared with control (A). Double-immunofluorescence staining of IGF-I (A and D) and GFAP (B and E) for detection of astrocytes showed that IGF-I positive cell could specifically co-express GFAP (arrowheads in C and F). Scale bars = 50μm.

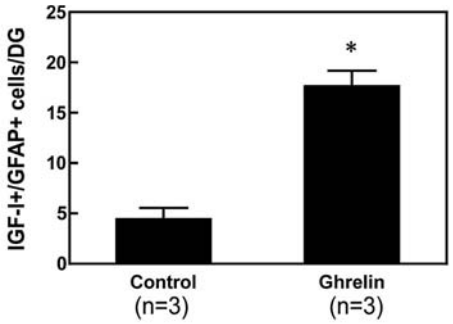

Fig. 3. Effect of ghrelin on the number of cells expressing both IGF-I and GFAP in the hippocampus of WT mice
Each bar represents mean ± S.D. from three animal experiments. *, $p < 0.01$ versus control.

(PBN) that connects to NTS and AP included in the sensory circumventricular organs (CVOs) [11]. Since CVOs lack the blood-brain barrier, these easily detect circulating hormones including ghrelin that are unable to cross the blood-brain barrier without a transport sys-

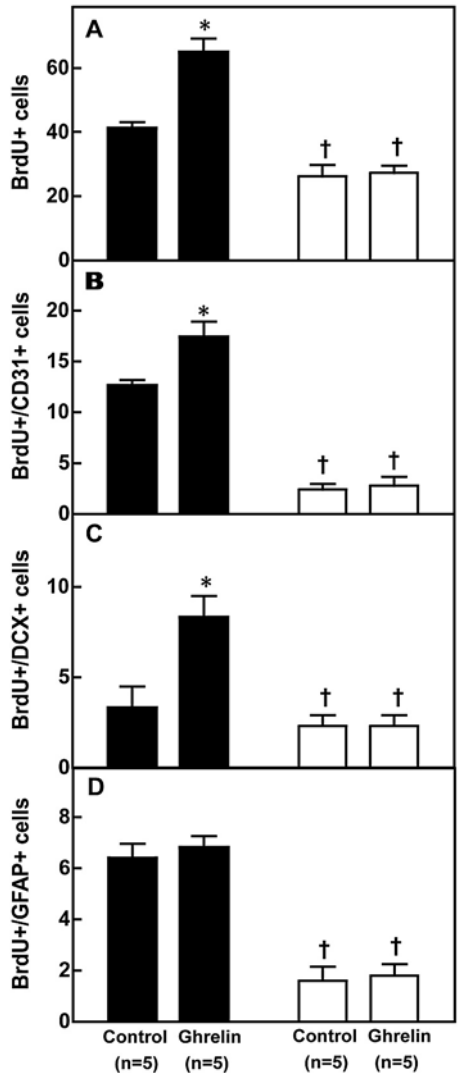

Fig. 4. Effect of ghrelin on the hippocampal angiogenesis and neurogenesis in WT mice and CGRP-/- mice

Angiogenesis was examined by the co-localization of the endothelial marker CD31and the proliferation marker BrdU. Neurogenesis was examined by the co-localization of the neuronal marker doublecortin（DCX）and the proliferation marker BrdU. All tissues were removed after the end of water maze. Each bar represents mean ± S.D. from five animal experiments. Solid bars, WT mice; open bars, CGRP-/-mice. *, $p<0.01$ versus ghrelin; †, $p<0.01$ versus WT mice.

tem[21, 22].

To analyze the mechanism and pathway of the relay system that leads to the increase of hippocampal IGF-I production in WT mice administered ghrelin, we determined *c-fos* expression in AP, NTS, PBN, and the hippocampus after administration of ghrelin. Increase of *c-fos* expression was observed in AP, NTS, PBN, and the hippocampus in WT mice, but not in CGRP-/- mice (Figs. 5 and 6).

Effect of ghrelin on spatial learning function in WT and CGRP-/- mice

To determine whether ghrelin improves cognitive function in mice by promoting CGRP release in the hippacampus, we examined the effect of ghrelin administration on spatial learn-

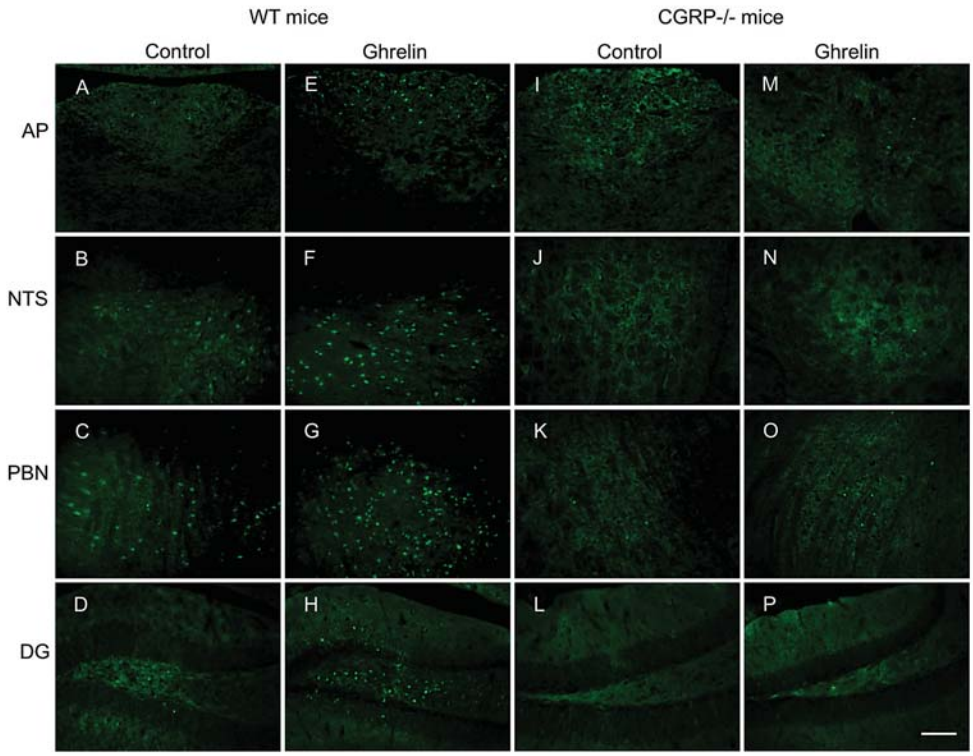

Fig. 5. Effect of ghrelin on *c-fos* expression in the brain nuclei of WT and CGRP-/- mice.
Ghrelin (10μg) injections took place twice per day for four consecutive days in WT mice (E, F, G, H) and CGRP-/- mice (M, N, O, P). Controls in WT mice (A, B, C, D) and those of CGRP-/- mice (I, J, K, L) were administered PBS. Immunohistochemial expression of *c-fos* in the area postrema (AP) indicated in A, E, I, and M; the solitary tract nucleus (NTS) indicated in B, F, J, and N; the medial parabrachial nucleus (PBN) indicated in C, G, K, and O; granular cell layer of the dentate gyrus (DG) indicated in D, H, L, and P. Five animals in each group were examined, and typical results are shown. Scale bars = 100μm.

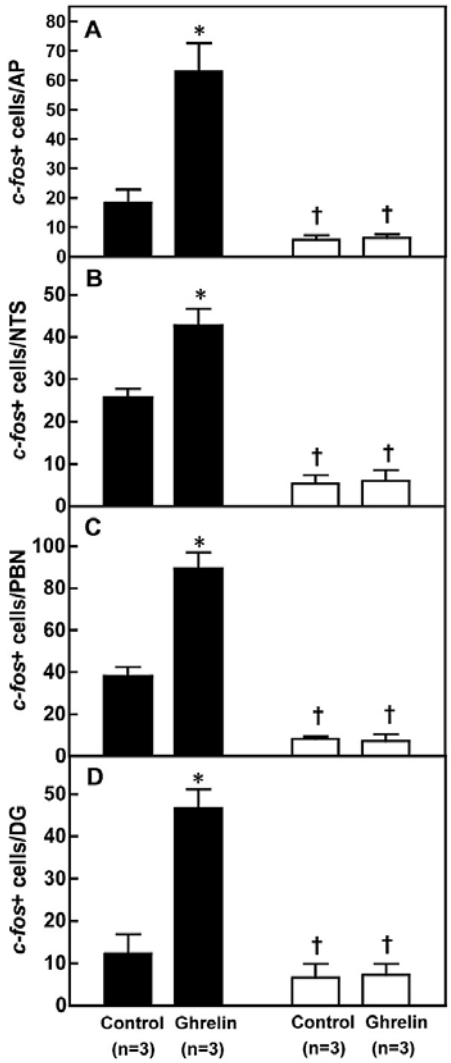

Fig. 6. Effect of ghrelin on the number of *c-fos*-positive cells in the area postrema (AP) (A), the solitary tract nucleus (NTS) (B), the medial parabrachial nucleus (PBN) (C), and the dentate gyrus (DG) (D) of WT mice and CGRP-/- mice.

Each bar represents mean ± S.D. from three experiments. *, $p < 0.01$ versus control; †, $p < 0.05$ versus WT mice.

ing function in WT and CGRP-/- mice by using the Morris water maze test for 5 consecutive days. In WT mice, the improvement in spatial learning on days 4 and 5 was significantly enhanced in animals treated with ghrelin for 4 days as compared with that in those not administered ghrelin (Fig. 7A). Ghrelin did not improve spatial learning through the 5 days in CGRP-/- mice (Fig. 7B). Similar effects were observed with time spent in the target area (probe test). Ghrelin improved the performance in the probe test in WT mice, but not in CGRP-/- mice (Fig. 8).

DISCUSSION

In the present study, we demonstrated that intraperitoneal administration of ghrelin increased hippocampal tissue levels of CGRP, IGF-I and IGF-I mRNA in WT mice. These observations strongly suggest that increases of tissue levels of IGF-I in WT mice administered

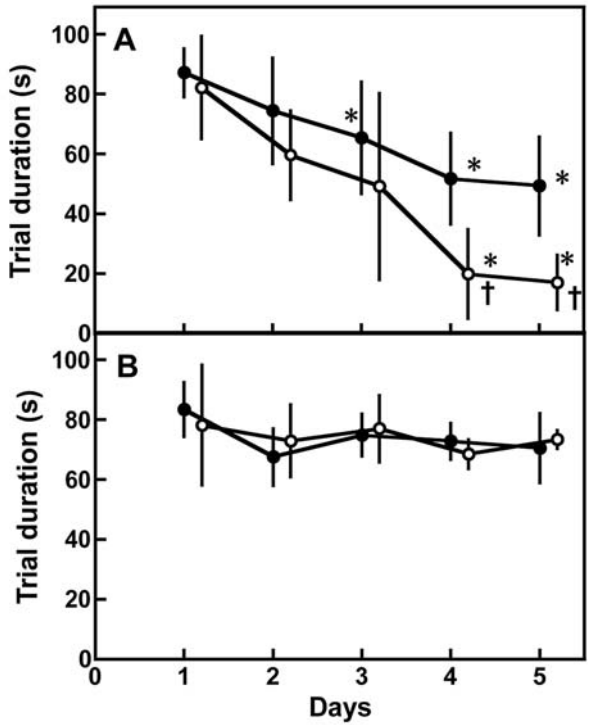

Fig. 7. Effects of ghrelin on spatial learning function in WT mice (A) and CGRP -/- mice (B)

Spatial learning was assessed by one trial day in the Morris water maze for 5 days. Each value is expressed as the mean ± S.D. derived from five animal experiments. Solid circles, control; open circles, ghrelin-treated mice. *, $p < 0.01$ versus day 1; †, $p < 0.01$ versus control.

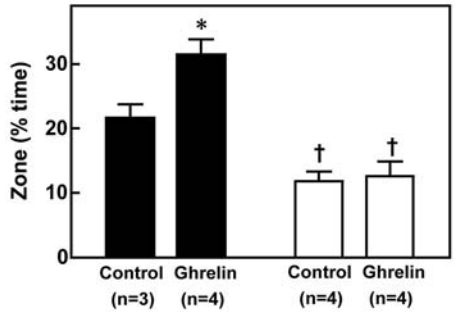

Fig. 8. Effects of ghrelin on spatial learning function (probe test) in WT mice and CGRP -/- mice.

Each value is expressed as the mean ± S.D. derived from three or four animal experiments. Solid bars, WT mice; open bars CGRP-/-mice. *, $p<0.01$ versus control; †, $p<0.05$ versus WT mice.

ghrelin may be explained at least in part by increasing its transcription in the hippocampus. Baseline hippocampal tissue levels of CGRP, IGF-I and IGF-I mRNA were significantly lower in CGRP-/- mice than in WT mice, and ghrelin did not increase tissue levels of CGRP, IGF-I and IGF-I mRNA in CGRP-/- mice, suggesting that ghrelin may promote CGRP release in the hippocampus, thereby increasing the hippocampal production of IGF-I in WT mice. Consistent with this notion, we previously reported that stimulation of periphery sensory neurons increased IGF-I production in the hippocampus of WT mice by increasing its transcription, but not in CGRP-/- mice[26].

Immunoreactivity of IGF-I was co-localized with that of the astrocyte marker GFAP in DG of WT mice administered ghrelin. This observation is consistent with a report showing that astrocytes are capable of producing IGF-I in the hippocampus[29]. CGRP has been shown to increase cAMP levels via CGRP receptor activation in astrocytes[30]. Because cAMP plays an important role in IGF-I production[31], ghrelin may promote CGRP release in the hippocampus, thereby increasing IGF-I production via increase of cAMP levels in astrocytes. Precisely which cells produce CGRP in the hippocampus of WT mice administered ghrelin is unknown at present.

The microtubule-associated protein doublecortin (DCX) is expressed by neuronal precursor cells and immature neurons in the brain, thus is being used as a marker for neurogenesis[32]. In the present study, ghrelin increased the number of BrdU+ cells, that of BrdU+ and DCX+ cells, and that of BrdU+ and CD31 + cells, but not that of BrdU+ and GFAP+ cells, in DG of WT mice. Ghrelin had no effect on the number of these cells in DG of CGRP-/- mice.

The number of CD31 + , DCX+, and GFAP+ cells in the BrdU-immunoreactive cells of DG in CGRP-/- mice was significantly lower than that of these cells in WT mice, suggesting that CGRP may play a critical role in neurogenesis and angiogenesis. Consistent with this hypothesis, stimulation of peripheral sensory neurons enhanced neurogenesis and angiogenesis by increasing the hippocampal production of IGF-I in WT mice, but not in CGRP-/- mice[33].

To analyze the mechanism and pathway of the relay system that leads to the increase of hippocampal IGF-I production in WT mice administered ghrelin, we determined *c-fos* expression in AP, NTS, PBN, and the hippocampus after administration of ghrelin. Increase of *c-fos* expression was observed in AP, NTS, PBN, and the hippocampus in WT mice, but not in CGRP-/- mice after administration of ghrelin. Both AP and NTS are included in the sensory CVOs that lack the blood-brain barrier[11]. Since ghrelin is unable to cross the blood-brain barrier without a transport system[21, 22], and nerve fibers in AP project to PBN where neural connection to the hippocampus is demonstrated[16,34], it is possible that ghrelin in the circulation stimulates AP and NTS, thereby transmitting this information to the hippocampus via PBN as a relay point. Expression of *c-fos* was not observed in these areas in CGRP-/- mice after ghrelin administration. CGRP is found in AP, NTS, PBN, and the hippocampus[16, 23, 34]. These observations strongly suggest that circulating ghrelin may stimulate neurons in AP and NTS, thereby transmitting the stimulatory information to PBN and the hippocampus, and that CGRP may function as a transmitter in this relay system. Consistent with this hypothesis, the ghrelin receptor GHSR-1 a is expressed in AP and NTS[35]. It is reported that the ghrelin can stimulate adrenocorticotropic hormone（ACTH）cells and significantly promote ACTH release[36]. Plasma ACTH is known related with Fos[37]. It is possible that ACTH can stimulate NTS and further research is needed.

Peripheral infusion of IGF-I was shown to selectively induce angiogenesis via a VEGF-dependent mechanism in the adult mouse brain[38]and neurogenesis in the adult rat hippocampus[13]. These observations strongly suggest that ghrelin may induce angiogenesis and neurogenesis by inducing IGF-I production via an increase in CGRP release in the mouse hippocampus. Because angiogenesis has been shown to provide a favorable environment for neuronal stem cell proliferation via activation of a VEGF-dependent mechanism[39], the hippocampal neurogenesis induced by ghrelin administration in WT mice may be mediated by angiogenesis. IGF-I exerts beneficial effects against the decline of cognitive function by in raising synaptic plasticity and neurogenesis in the hippocampus[13], suggesting that ghrelin may improve the cognitive function by inducing IGF-I production through promoting CGRP release in the mouse hippocampus. Consistent with this hypothesis, ghrelin significantly improved spatial learning function in WT mice, but not in CGRP-/- mice. These observations suggest that stimulation of neurons in CVOs with ghrelin may increase IGF-I production by promoting

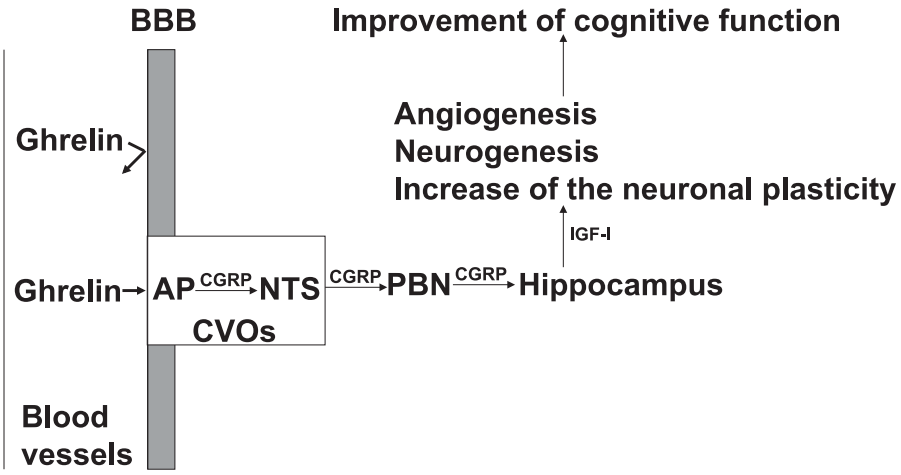

Fig. 9. A diagram of the possible mechanism by which ghrelin improves cognitive function

AP, the area postrema; BBB, blood-brain barrier; CVOs, circumventricular organs; NTS, the nucleus tractus solitarius; PBN, the parabrachial nuclei

CGRP release in the mouse hippocampus, thereby improving cognitive function. Since newly generated neurons are functionally integrated and begin modifying active hippocampal circuits spend approximately 2-4 weeks[40], the ghrelin induced improvement of cognitive function we observed appeared to mainly dependent on enhancement on synaptic transmission and plasticity by IGF-I in the hippocampus.

Taken together, observations in the present study strongly suggest that ghrelin may stimulate neurons in CVOs, thereby increasing CGRP release in the hippocampus, and CGRP may in turn increase IGF-I production, thereby promoting cognitive function in mice. Figure 9 depicts the possible mechanism by which ghrelin improves cognitive function based on findings obtained in the present study.

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