Multiple signaling pathways mediate ghrelin-induced proliferation of hippocampal neural stem cells

Hyunju Chung, Endan Li[1], Yumi Kim[1], Sehee Kim[1] and Seungjoon Park[1]

Department of Core Research Laboratory, Clinical Research Institute, Kyung Hee University Hospital at Gangdong, Seoul, Korea
[1]Department of Pharmacology and Medical Research Center for Bioreaction to ROS and Biomedical Science Institute, School of Medicine, Kyung Hee University, Seoul 130-701, Korea

Correspondence should be addressed to S Park
Email
sjpark@khu.ac.kr

Abstract

Ghrelin, an endogenous ligand for the GH secretagogue receptor (GHS-R) receptor 1a (GHS-R1a), has been implicated in several physiologic processes involving the hippocampus. The aim of this study was to investigate the molecular mechanisms of ghrelin-stimulated neurogenesis using cultured adult rat hippocampal neural stem cells (NSCs). The expression of GHS-R1a was detected in hippocampal NSCs, as assessed by western blot analysis and immunocytochemistry. Ghrelin treatment increased the proliferation of cultured hippocampal NSCs assessed by BrdU incorporation. The exposure of cells to the receptor-specific antagonist D-Lys-3-GHRP-6 abolished the proliferative effect of ghrelin. By contrast, ghrelin showed no significant effect on cell differentiation. The expression of GHS-R1a was significantly increased by ghrelin treatment. The analysis of signaling pathways showed that ghrelin caused rapid activation of ERK1/2 and Akt, which were blocked by the GHS-R1a antagonist. In addition, ghrelin stimulated the phosphorylation of Akt downstream effectors, such as glycogen synthase kinase (GSK)-3β, mammalian target of rapamycin (mTOR), and p70^{S6K}. The activation of STAT3 was also caused by ghrelin treatment. Furthermore, pretreatment of cells with specific inhibitors of MEK/ERK1/2, phosphatidylinositol-3-kinase (PI3K)/Akt, mTOR, and Jak2/STAT3 attenuated ghrelin-induced cell proliferation. Taken together, our results support a role for ghrelin in adult hippocampal neurogenesis and suggest the involvement of the ERK1/2, PI3K/Akt, and STAT3 signaling pathways in the mediation of the actions of ghrelin on neurogenesis. Our data also suggest that PI3K/Akt-mediated inactivation of GSK-3β and activation of mTOR/p70^{S6K} contribute to the proliferative effect of ghrelin.

Key Words

- ghrelin
- hippocampus
- progenitor
- proliferation
- signaling pathways

Journal of Endocrinology (2013) **218**, 49–59

Introduction

Ghrelin, a 28-amino acid peptide hormone mainly produced in the stomach, has been shown to stimulate GH release by activating the GH secretagogue (GHS) receptor 1a (GHS-R1a; Kojima *et al*. 1999). Initial studies have shown that ghrelin acts primarily at the anterior pituitary and hypothalamus to stimulate GH release and food intake to regulate energy homeostasis and body weight (Date *et al*. 2000). In addition, ghrelin exerts numerous peripheral effects including direct effects on exocrine and endocrine pancreatic functions, carbohydrate metabolism, the cardiovascular system, gastric secretion, stomach motility, and sleep (Van der Lely

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

et al. 2004, Ghigo *et al.* 2005, Kojima & Kangawa 2005). Numerous studies have indicated that ghrelin has multiple nonendocrine functions in the CNS to control neuronal function and consequently influence diverse brain functions, such as learning and memory (Diano *et al.* 2006), anxiety and depression (Carlini *et al.* 2004, Lutter *et al.* 2008), reward and motivation (Naleid *et al.* 2005, Abizaid *et al.* 2006, Jiang *et al.* 2006), and neuroprotection (Jiang *et al.* 2006, 2008, Chung *et al.* 2007, Miao *et al.* 2007, Hwang *et al.* 2009, Moon *et al.* 2009*a*, Lee *et al.* 2010*a*,*b*).

Neurogenesis, a process of generating functionally integrated neurons from progenitor cells (i.e. proliferation, survival, differentiation, and migration of neuronal precursor cells), persists into adulthood in several species, including humans, in the subventricular zone of the lateral ventricle and in the subgranular zone of the dentate gyrus (DG) in the hippocampus (Ming & Song 2005, Zhao *et al.* 2008). There has been much attention focused on neurogenesis in the hippocampus in the normal brain because this structure is important in the process of learning, memory, and emotional responses (Zhao *et al.* 2008). Several endogenous growth factors, such as fibroblast growth factor-2, IGF1, and vascular endothelial growth factor, improve cognitive function either by direct effects on the generation of new neurons or indirectly through neurotrophic effects that promote the survival of new neurons in the hippocampus (Grote & Hannan 2007). However, the endogenous factors that regulate the proliferation of progenitor cells in the adult hippocampus need to be further clarified.

Several studies have demonstrated that ghrelin enhances neurogenesis. Initially, ghrelin was known to increase neurogenesis in the rat fetal spinal cord (Sato *et al.* 2006) and the nucleus of the solitary tract (Zhang *et al.* 2005) and the dorsal motor nucleus of vagus (Zhang *et al.* 2004) in adult rats. We previously reported that systemic administration of ghrelin induces hippocampal neurogenesis in adult mice (Moon *et al.* 2009*b*). Moreover, in our recent study (Li *et al.* 2013), we found that ghrelin knockout mice showed lower numbers of progenitor cells in the DG of the hippocampus, while ghrelin treatment restored the numbers of progenitor cells to those of the wild-type controls. In addition, it has been reported that ghrelin increases the cellular proliferation of cultured adult rat hippocampal progenitor cells through the activation of MAPK pathways (Johansson *et al.* 2008). Collectively, these data indicate that ghrelin may promote the proliferation of hippocampal progenitor cells and thereby act as a neurogenic agent. However, the molecular mechanisms underlying the proliferative effect of ghrelin in adult hippocampal progenitor cells are still unclear. Therefore, in this study, we characterized the possible signaling mechanisms by which ghrelin exerts its effects on neurogenesis in cultured adult rat hippocampal neural stem cells (NSCs).

Materials and methods

Materials

Rat ghrelin was obtained from Peptides International (Louisville, KT, USA). D-Lys-3-GHRP-6 was purchased from Bachem (Torrance, CA, USA). NSC expansion media, DMEM/F12, and B27 supplement were obtained from Gibco/Invitrogen. B-27 is an optimized serum substitute developed for low-density plating and long-term viability and growth of CNS neurons (Brewer *et al.* 1993). PD98059, U0126, LY294002, rapamycin, and cucurbitacin I were obtained from Tocris (Ellisville, MO, USA) and Akt inhibitor VIII was procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All tissue culture reagents were obtained from Gibco/Invitrogen, and all other reagents were obtained from Sigma unless otherwise indicated.

Adult rat hippocampal NSC cultures and treatments

Adult rat hippocampal NSCs were obtained from Chemicon (Catalog No. SCR022, Billerica, MA, USA). These cells are ready-to-use primary NSCs isolated from the hippocampus of adult Fisher 344 rats. They were grown in a NSC expansion medium containing DMEM/F12 with L-glutamine, B27 supplement, 1× solution of penicillin, streptomycin and fungizone, and basic FGF (bFGF, 20 ng/ml). Tissue culture plastic- or glasswares that were used to culture hippocampal NSCs were coated with poly-L-ornithine (10 μg/ml) and laminin (5 μg/ml). The hippocampal NSCs were maintained at 37 °C in a 5% CO_2 humidified incubator and with a complete change of media containing fresh bFGF every other day and passaged once every 5–6 days. To determine whether ghrelin stimulates the proliferation of hippocampal NSCs, cells were treated with increasing doses of ghrelin (1 nM to 10 μM) for 48 h. As dose–response experiments showed that 100 nM ghrelin was the lowest dose with the maximum response, this dose of ghrelin was used in subsequent experiments. To investigate the effect of ghrelin on the expression of its receptor, cells were treated with ghrelin (100 nM) for 2, 4, 8, and 24 h. Experiments were also performed by incubating the cells with the following pharmacological inhibitors: 20 μM PD98059 for

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

0.5 h, 10 μM U0126 for 0.5 h, 20 μM LY294002 for 1 h, 100 nM Akt inhibitor VIII for 1 h, 200 nM rapamycin for 1 h, or 1 nM cucurbitacin I for 0.5 h. Cell proliferation was assessed by performing immunocytochemical staining for BrdU and counting the number of BrdU-positive cells. To investigate the effect of ghrelin on the phosphorylation of ERK1/2, Akt, glycogen synthase kinase (GSK)-3β, mammalian target of rapamycin (mTOR), $p70^{S6K}$, and STAT3, cells were treated with ghrelin (100 nM) for 15, 30, 60, and 120 min and assayed by western blot analysis as described below. To determine whether the effects of ghrelin on cell proliferation and phosphorylation were mediated via its receptor, GHS-R1a, cells were pretreated with D-Lys-3-GHRP-6 (100 μM) or a vehicle (saline) for 1 h before the treatment with ghrelin (100 nM). All experiments were performed three times in duplicate.

Detection of GHS-R1a expression in adult rat hippocampal NSCs

We performed immunocytochemistry to detect the protein expression of GHS-R1a in hippocampal NSCs. Briefly, cells were fixed with 4% paraformaldehyde (PFS; Sigma) in PBS for 30 min at room temperature. After blocking with 3% normal goat serum (Vector Laboratories, Burlingame, CA, USA) and 1% BSA (Sigma), the slides were incubated with primary antibodies to nestin (1:500; Millipore, Temecula, CA, USA) and GHS-R1a (1:500; Santa Cruz Biotechnology) overnight at 4 °C. After washes, the slides were incubated with a secondary antibody (Alexa Fluor 488 donkey anti-goat IgG, 1:400; Invitrogen) at room temperature for 1.5 h. Cells were counterstained with DAPI before mounting, and images were acquired by the Carl Zeiss LSM 700 Meta confocal microscope. To determine the specificity of the antibody used in this study, we performed immunocytochemical staining using HepG2 cells, which are known to not express GHS-R1a (Thielemans *et al*. 2007). The slides incubated without the primary antibody for GHS-R1a were also included as negative controls. We also performed western blot analysis to detect the protein expression of GHS-R1a in adult rat hippocampal NSCs as described below.

Evaluation of cell proliferation and differentiation

Experiments for cell proliferation were performed in six-well chamber slides. Cells were seeded at a density of 8×10^4 cells/ml of neuronal expansion media containing bFGF. After 24 h, the media were replaced with fresh media with ghrelin (1 nM to 10 μM) or a vehicle and incubated for 48 h. Cells were treated with BrdU (10 μM) for 4 h and then fixed with 4% PFA. Four random fields for each well were chosen under the 20× objective, and the total number of BrdU-labeled cells was counted by a person who was blind to the treatment.

To examine whether ghrelin regulates the differentiation of hippocampal NSCs, cells were treated with ghrelin (100 nM) or a vehicle for 48 h in neural expansion media without bFGF. Then, cells were incubated with BrdU (10 μM) for 4 h. Cells were incubated for an additional 8 days, and during this time, the media were changed every other day. Double-labeling immunocytochemistry for BrdU and the neuronal marker Tuj1 (1:1000; Abcam, Cambridge, UK) or the glial marker GFAP (1:1000; Zymed Laboratories, Carlsbad, CA, USA) was performed after the cells were fixed in 4% PFA. Cells were rinsed with PBS, followed by incubation with a secondary antibody (Alexa Fluor 546 goat anti-rabbit IgG, 1:400; Invitrogen) for 4 h at room temperature.

For immunocytochemical detection of BrdU in hippocampal NSCs, the fixed cells were incubated in 2 M HCl and 0.3% Triton X-100 for 30 min followed by incubation in 0.1 M boric acid (pH 8.0) for 10 min. Cells were incubated in a blocking solution (0.3% Triton X-100, 1% BSA, and 3% normal goat serum in PBS) for 2 h. After overnight incubation with a primary antibody (mouse anti-BrdU, 1:400; Roche) at 4 °C, cells were rinsed with PBS, followed by incubation with a secondary antibody (Alexa Fluor 488 goat anti-mouse IgG, 1:400, Invitrogen) for 4 h at room temperature. BrdU-labeled cells were visualized by a fluorescence microscope.

Western blot analysis

Cells were lysed in a buffer containing 20 mM Tris–HCl (pH 7.4), 1 mM EDTA, 140 mM NaCl, 1% (w/v) Nonidet P-40, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 50 mM NaF, and 10 μg/ml aprotinin. Cell lysates were separated by 12% SDS–PAGE and electrotransferred onto polyvinylidene difluoride membranes (Bio-Rad). The membranes were soaked in a blocking buffer (1× Tris-buffered saline, 1% BSA, and 1% nonfat dry milk) for 1 h and incubated overnight at 4 °C with the primary antibodies against ERK1/2, phospho-ERK1/2 on Thr202/Tyr204, STAT3, phospho-STAT3 on Tyr705, Akt, phospho-Akt on Thr308, GSK-3β, phospho-GSK-3β on Ser9, mTOR, phospho-mTOR on Ser2448, $p70^{S6K}$, phospho-$p70^{S6K}$ on Thr389 (Cell Signaling, Danvers, MA, USA; 1:1000), and GHS-R1a (Santa Cruz Biotechnology; 1:1000). Blots were developed using a peroxidase-conjugated anti-rabbit IgG and a chemiluminescent detection system (Santa Cruz

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

Biotechnology). The bands were visualized using a ChemicDoc XRS system (Bio-Rad) and quantified using Quantity One imaging software (Bio-Rad).

Statistical analysis

Data are presented as mean±S.E.M. of three different experiments (each experiment was performed in duplicate). Statistical analysis between groups was performed using one-way ANOVA and the Holm–Sidak method for multiple comparisons using SigmaStat for Windows Version 3.10 (Systat Software, Inc., Point Richmond, CA, USA). $P<0.05$ was considered statistically significant.

Results

Expression of GHS-R1a in adult rat hippocampal NSCs

To determine whether adult rat hippocampal NSCs express the ghrelin receptor GHS-R1a, we examined the protein expression of GHS-R1a in cultured hippocampal NSCs by western blot analysis and immunocytochemistry. Results obtained from the western blot analysis revealed that GHS-R1a protein was present in these cells (Fig. 1A). Total protein extracted from the hypothalamus was used as a positive control. We failed to identify the expression of GHS-R1a in HepG2 cells, which are known to not express GHS-R1a (Thielemans *et al*. 2007), indicating the specificity of the antibody. The presence of GHS-R1a in hippocampal NSCs was further confirmed by immunocytochemical staining with antibodies against GHS-R1a and nestin. Figure 1B shows that GHS-R1a immunoreactivity was clearly observed in cultured hippocampal NSCs. We found no GHS-R1a immunoreactivity in HepG2 cells. In addition, no labeling was present in the primary antibody control (Fig. 1C).

Effect of ghrelin on cell proliferation and differentiation in adult rat hippocampal NSCs

To determine whether ghrelin has a direct role in the proliferation of adult rat hippocampal NSCs, cells were incubated with different concentrations of ghrelin prior to BrdU labeling. The analysis of the number of BrdU-labeled cells showed an increase in cell proliferation in a concentration-dependent manner (Fig. 2A and B). The maximal response was observed with the dose of 100 nM (143% of the vehicle-treated control); therefore, this dose was used in subsequent experiments. To determine whether the proliferative effect of ghrelin is mediated by its receptor GHS-R1a, hippocampal NSCs were treated with the ghrelin receptor-specific antagonist. The exposure of cells to D-Lys-GHRH-6 (100 μM) completely abolished the proliferative effect of ghrelin (Fig. 2C and D).

To determine whether ghrelin affects the differentiation of adult rat hippocampal NSCs, cells were treated with ghrelin for 8 days following BrdU labeling without bFGF in the media to allow differentiation. Ghrelin treatment showed no significant effect on the number of BrdU-labeled cells and the percentages of BrdU-labeled cells that were positive for the neuronal marker Tuj1 or the glial marker GFAP (Fig. 2E and F).

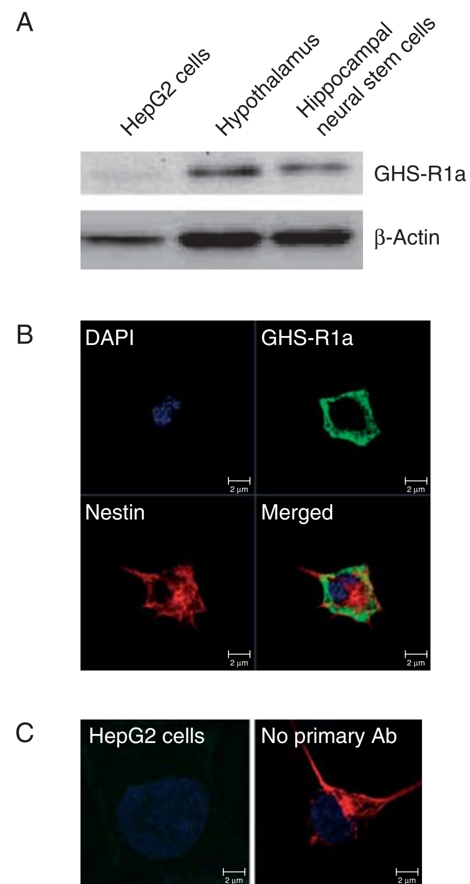


Figure 1
Expression of GHS-R1a in cultured adult rat hippocampal NSCs. (A) GHS-R1a protein expression assessed by western blot analysis. Total protein extracted from the hypothalamus was included as a positive control. Total protein from HepG2 cells was used as a negative control. (B) GHS-R1a immunoreactivity in adult rat hippocampal NSCs. Cells were incubated with primary antibodies to GHS-R1a and nestin. Cells were counterstained with DAPI, and images were captured using confocal microscopy; scale bar, 2 μm. (C) Lack of GHS-R1a immunoreactivity in HepG2 cells (left). The slide incubated without the primary antibody (Ab) for GHS-R1a was used as a negative control (right); scale bar, 2 μm.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

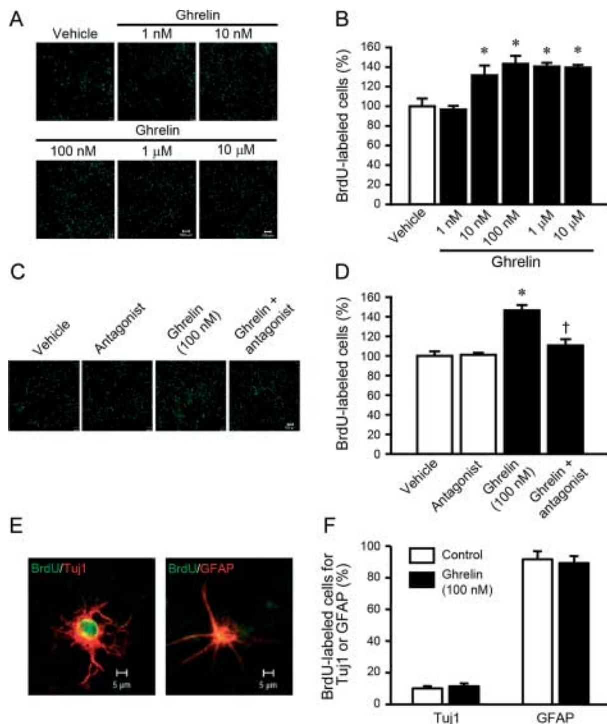


Figure 2
Effects of ghrelin on the proliferation and differentiation of cultured adult rat hippocampal NSCs. (A and B) Cells were treated with various concentrations of ghrelin (1 nM–10 µM) for 48 h and labeled with BrdU (10 µM) in the last 4 h of incubation. (A) Representative microscopic images showing BrdU-labeled adult rat hippocampal NSCs. Scale bar represents 100 µm. (B) Quantitative analysis showed that the number of BrdU-labeled cells was increased by ghrelin treatment at concentrations of 10 nM to 10 µM when compared with the control. (C and D) Cells were preincubated with a vehicle or the GHS-R1a antagonist D-Lys-3-GHRP-6 (100 µM) for 1 h and then treated with the vehicle or ghrelin (100 nM) for 48 h. (C) Representative microscopic images showing BrdU-labeled adult rat hippocampal NSCs. Scale bar represents 100 µm. (D) Quantitative analysis revealed that the exposure of cells to the ghrelin receptor antagonist abolished the proliferative effect of ghrelin. (E and F) Cells were treated with a vehicle or ghrelin (100 nM) for 48 h and labeled with BrdU (10 µM) in the last 4 h of incubation and were allowed to differentiate for 8 days before fixation for immunocytochemical processing. (E) Representative confocal microscopic images showing that BrdU-labeled (green) cells differentiated into neuronal (Tuj1-positive in red) or glial (GFAP-positive in red) cells. Scale bar represents 5 µm. (F) Quantitative analysis showed that the percentage of BrdU-labeled cells that were positive for Tuj1 or GFAP was not significantly altered by ghrelin treatment. The data are expressed as the mean ± S.E.M. of three different experiments (each experiment was performed in duplicate). *$P<0.05$ vs the vehicle-treated control and †$P<0.05$ vs ghrelin-treated cells.

Effect of ghrelin on GHS-R1a protein levels in adult rat hippocampal NSCs

The treatment of cells with ghrelin increased GHS-R1a protein levels in a time-dependent manner (Fig. 3). Ghrelin-induced increase of the expression of GHS-R1a peaked between 4 and 8 h and lasted for 24 h. These results suggest that GHS-R1a protein levels were affected by ghrelin treatment in these cells.

Ghrelin stimulates the proliferation of adult rat hippocampal NSCs by activating MEK/ERK1/2, phosphatidylinositol-3-kinase/Akt and Jak2/STAT3 signaling pathways

It has been demonstrated that ghrelin activates multiple signal transduction pathways, including ERK1/2 (Chung *et al*. 2007), phosphatidylinositol-3-kinase (PI3K)/Akt (Chung *et al*. 2008), and Jak2/STAT3 pathways (Park *et al*. 2008), which are important signaling pathways for the proliferation of adult hippocampal progenitor cells (Weeber & Sweatt 2002, Hao *et al*. 2004, Yoshimatsu *et al*. 2006, Peltier *et al*. 2007). To determine the signaling pathways that can be activated by ghrelin in adult rat hippocampal NSCs, the phosphorylation of ERK1/2, Akt, and STAT3 was examined by western blot analysis after ghrelin treatment. The treatment of cells with ghrelin activated ERK1/2, Akt, and STAT3 in a time-dependent manner (Fig. 4A, C, and E). Ghrelin-induced activation of ERK1/2, Akt, and STAT3 peaked between 30 and 60 min and lasted for 120 min. In order to determine whether ghrelin-induced phosphorylation of ERK1/2, Akt, and STAT3 is mediated by its receptor GHS-R1a, hippocampal NSCs were treated with the ghrelin receptor-specific antagonist. The exposure of cells to D-Lys-GHRH-6 (100 µM) significantly inhibited the stimulatory effects of ghrelin on the phosphorylation of ERK1/2, Akt, and STAT3 (Fig. 4B, D, and F).

We also investigated the effect of ghrelin on the Akt downstream effectors, such as GSK-3β, mTOR, and $p70^{S6K}$, which are known to regulate cell proliferation (Ryu *et al*. 2003, Song *et al*. 2005, Adachi *et al*. 2007, Han *et al*. 2008). The phosphorylation of GSK-3β was increased after 15 min of ghrelin treatment and lasted for 120 min (Fig. 5A). Moreover, ghrelin caused a rapid and strong phosphorylation of mTOR (Fig. 5C) and $p70^{S6K}$ (Fig. 5E). The treatment of cells with D-Lys-GHRH-6 significantly attenuated the stimulatory effects of ghrelin on the phosphorylation of GSK-3β, mTOR, and $p70^{S6K}$ (Fig. 5B, D, and F).

To further determine whether the activation of MEK/ERK1/2, PI3K/Akt, and Jak2/STAT3 signaling pathways mediates the effect of ghrelin on the proliferation of adult rat hippocampal NSCs, cells were exposed to MEK1 inhibitor PD98059, MEK1/2 inhibitor U0126, PI3K inhibitor LY294002, Akt inhibitor VIII, mTOR inhibitor rapamycin, or Jak2/STAT3 inhibitor cucurbitacin I,

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

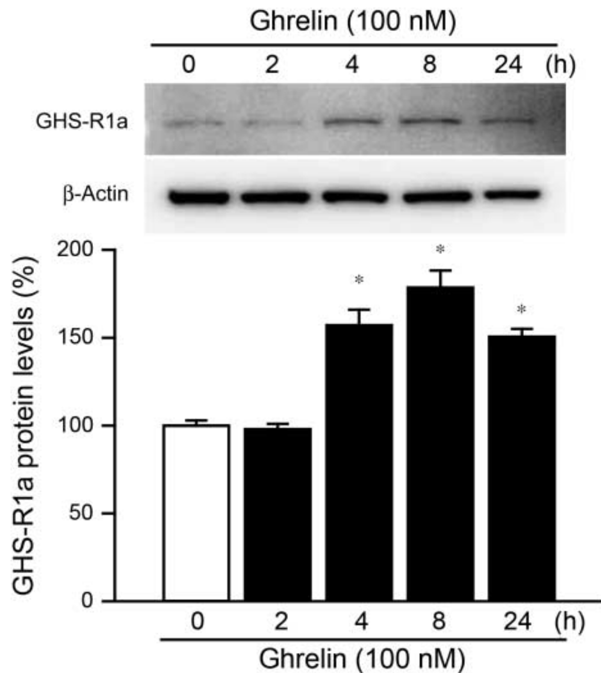


Figure 3
Effects of ghrelin on GHS-R1a protein levels in cultured adult rat hippocampal NSCs. Cells were treated with 100 nM ghrelin for 2, 4, 8, and 24 h. Protein lysates were prepared and assayed by western blot analysis using anti-GHS-R1a and anti-β-actin antibodies. The GHS-R1a band intensity was normalized to the β-actin band intensity. Representative western blot images are shown in the upper insets. The data are expressed as the mean ± S.E.M. of three different experiments (each experiment was performed in duplicate). $*P<0.05$ vs the control.

followed by ghrelin treatment. We found that all these inhibitors significantly blocked the proliferative effects of ghrelin in hippocampal NSCs (Fig. 6A and B).

Discussion

It is well known that ghrelin plays important roles in GH release, food intake, body weight regulation, and glucose homeostasis. In addition to its importance in energy metabolism, the abundant expression of GHS-R1a in brain regions outside the hypothalamus suggests its significance in neuronal function. A plethora of evidence from the last decade of research suggests that ghrelin acts in the CNS to control neuronal function and subsequently has a profound influence on various brain functions (Andrews 2011). In the current study, we demonstrated that ghrelin increases the proliferation of adult rat hippocampal NSCs via the activation of GHS-R1a but not the differentiation of cells. We also demonstrated that ghrelin treatment upregulates the expression of GHS-R1a. The proliferative effects of ghrelin were dependent on the activities of ERK1/2, PI3K/Akt, and Jak2/STAT3 signaling pathways. Ghrelin-induced stimulation of the PI3K/Akt pathway resulted in the inactivation of GSK-3β and the activation of mTOR/$p70^{S6K}$ in these cells.

Ghrelin exerts its effects by activating the only functional receptor, GHS-R1a, which is a G-protein-coupled 7 transmembrane receptor. The GHS-R1a belongs to a family of receptors operating via the Gq-phospholipase C pathway, and it was first cloned from the pituitary gland and hypothalamus (Howard *et al.* 1996). This receptor is also highly expressed in brain regions outside the hypothalamus, including the DG of the hippocampus

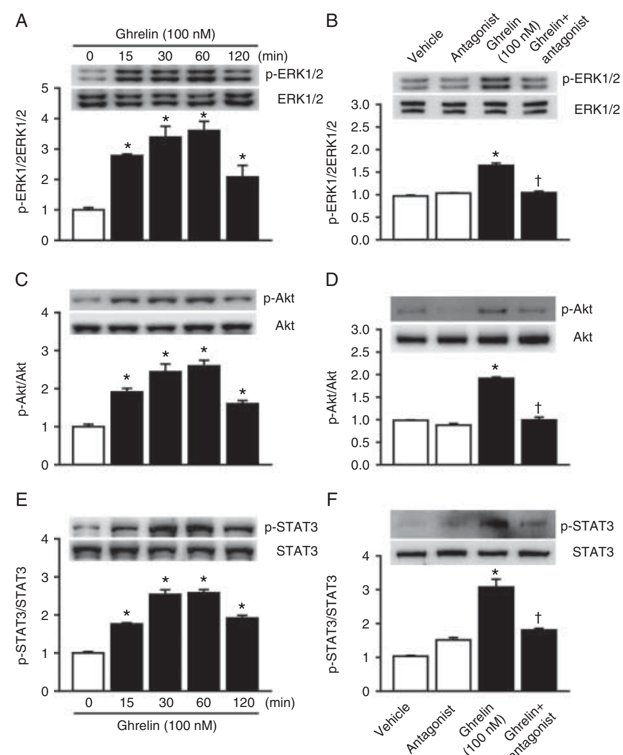


Figure 4
Effects of ghrelin on the phosphorylation of ERK1/2, Akt, and STAT3 in cultured adult rat hippocampal NSCs. (A, C, and E) Time-course of ghrelin-induced phosphorylation of ERK1/2, Akt, and STAT3. Cells were treated with 100 nM ghrelin for 15, 30, 60, and 120 min. (B, D, and F) Cells were preincubated with a vehicle or the GHS-R1a antagonist D-Lys-3-GHRP-6 (100 μM) for 1 h followed by treatment with the vehicle or ghrelin (100 nM) for 30 min. Protein lysates were prepared and assayed by western blot analysis using specific anti-phospho-ERK1/2 (Thr202/Tyr204) and anti-ERK1/2 antibodies (A and B), anti-phospho-Akt (Ser473) and anti-Akt antibodies (C and D), and anti-phospho-STAT3 (Tyr705) and anti-STAT3 antibodies (E and F). The band intensities of phospho-forms were normalized to the band intensities of total-forms respectively, and they are expressed as relative band intensities. The data are expressed as the mean ± S.E.M. of three different experiments (each experiment was performed in duplicate). $*P<0.05$ vs the control and $^{\dagger}P<0.05$ vs ghrelin-treated cells.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

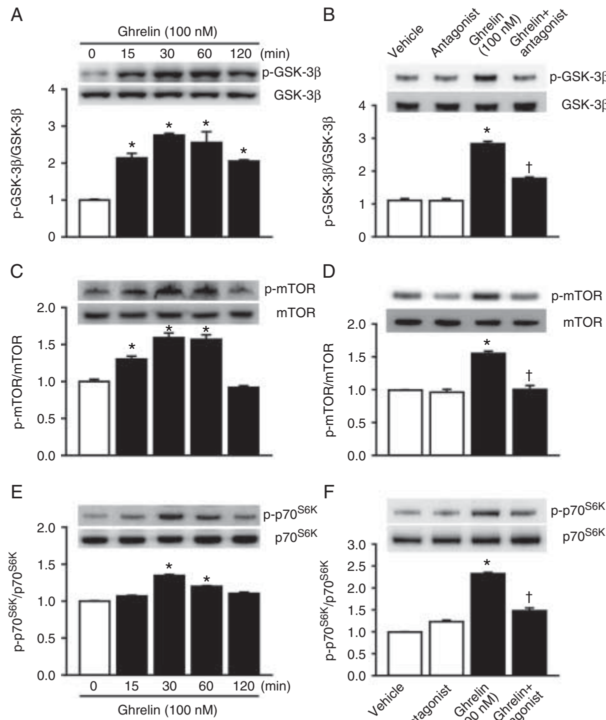
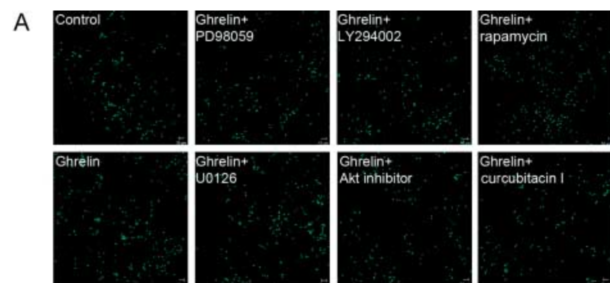


Figure 5
Effects of ghrelin on the phosphorylation of GSK-3β, mTOR, and $p70^{S6K}$ in cultured adult rat hippocampal NSCs. (A, C, and E) Time-course of ghrelin-induced phosphorylation of GSK-3β, mTOR, and $p70^{S6K}$. Cells were treated with 100 nM ghrelin for 15, 30, 60, and 120 min. (B, D, and F) Cells were preincubated with a vehicle or the GHS-R1a antagonist D-Lys-3-GHRP-6 (100 μM) for 1 h followed by treatment with the vehicle or ghrelin (100 nM) for 30 min. Protein lysates were prepared and assayed by western blot analysis using specific anti-phospho-GSK-3β (Ser9) and anti-GSK-3β antibodies (A and B), anti-phospho-mTOR (Ser2448) and anti-mTOR antibodies (C and D), and anti-phospho-$p70^{S6K}$ (Thr389) and anti-$p70^{S6K}$ antibodies (E and F). The band intensities of phospho-forms were normalized to the band intensities of total-forms respectively, and they are expressed as relative band intensities. The data are expressed as the mean ± S.E.M. of three different experiments (each experiment was performed in duplicate). *$P<0.05$ vs the control and †$P<0.05$ vs ghrelin-treated cells.

(Guan *et al.* 1997). In the current study, we clearly showed that the ghrelin receptor was expressed in cultured adult rat hippocampal NSCs at the protein levels, which were assessed by two independent methods (western blot analysis and immunocytochemistry). GHS-R1a was also found in hippocampal progenitor cells in adult mice (Moon *et al.* 2009*b*). The effect of ghrelin on the proliferation of adult rat hippocampal NSCs appears to be mediated through the activation of GHS-R1a because the treatment of the receptor-specific antagonist D-Lys-3-GHRP-6 completely blocked the proliferative effect of ghrelin. Similar findings were observed in neuronal cells exposed to oxygen–glucose deprivation (Chung *et al.* 2007, 2008). The receptor-mediated effect of ghrelin in these cells is further supported by the findings that ghrelin-induced phosphorylation of ERK1/2, Akt, STAT3, GSK-3β, mTOR, and $p70^{S6K}$ was significantly attenuated when cells were pretreated with D-Lys-3-GHRP-6. However, it should be noted that the GHS-R1a-independent effects of ghrelin have been reported previously (Baldanzi *et al.* 2002, Delhanty *et al.* 2006, Johansson *et al.* 2008).

Our recent report showed that differentiation is also affected by ghrelin, because a decrease was observed not only in the number of BrdU-positive cells but also in the



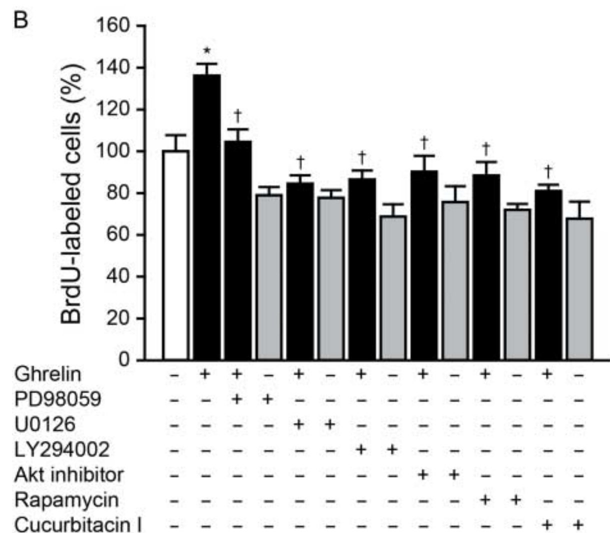


Figure 6
Effects of the blockade of MEK/ERK1/2, PI3K/Akt/mTOR, and Jak2/STAT3 signaling pathways on ghrelin-induced proliferation of adult rat hippocampal NSCs. Cells were preincubated with 20 μM PD98059 for 1 h, 10 μM U0126 for 0.5 h, 20 μM LY294002 for 1 h, 100 nM Akt inhibitor for 1 h, 200 nM rapamycin for 1 h, or 1 nM cucurbitacin I for 0.5 h and then treated with a vehicle or ghrelin (100 nM) for 48 h and labeled with BrdU (10 μM) in the last 4 h of incubation. (A) Representative microscopic images showing BrdU-labeled hippocampal NSCs. Scale bar represents 100 μm. (B) Quantitative analysis showed that all inhibitors blocked the proliferative effects of ghrelin. The data are expressed as the mean ± S.E.M. of three different experiments (each experiment was performed in duplicate). *$P<0.05$ vs the vehicle-treated control and †$P<0.05$ vs ghrelin-treated cells.

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

Journal of Endocrinology

fraction of newly generated neurons in ghrelin knockout mice after 28 days of BrdU administration, which were increased by ghrelin replacement (Li *et al*. 2013). However, our *in vitro* data indicated that ghrelin showed no effect on the number of BrdU-labeled cells and Tuj1- or GFAP/BrdU-double positive cell fraction, suggesting that the effect of ghrelin on the differentiation of newborn cells may require GH and/or IGF1 increased by ghrelin treatment. Indeed, IGF1 is known to increase neuronal differentiation in the DG of the adult hippocampus (Aberg *et al*. 2000).

In the current study, we found that ghrelin increased the GHS-R1a protein levels in cultured adult rat hippocampal NSCs. This finding was supported by the evidence that the mRNA levels of GHS-R1a in the hypothalamic arcuate nucleus were increased by ghrelin treatment in rats (Nogueiras *et al*. 2004). Ghrelin-induced upregulation of GHS-R1a was also found in middle cerebral artery-occluded rats (Miao *et al*. 2007). In this study, the protein expression of GHS-R1a was clearly observed in cultured adult rat hippocampal NSCs assessed by two different methods (western blot analysis and immunocytochemical staining). However, it should be noted that Johansson *et al*. (2008) did not find the mRNA of the ghrelin receptor in these cells as determined by RT-PCR analysis, although they showed proliferative response to acylated ghrelin.

One may bring up the issue of the specificity of the GHS-R1a antibody used in this study. Therefore, we validated the specificity of the antibody. HepG2 cells were used as a negative control, because these cells are known to not express GHS-R1a (Thielemans *et al*. 2007). Immunocytochemical staining and western blot analysis revealed that GHS-R1a protein was not expressed in HepG2 cells. We also found no labeling in the primary or secondary antibody controls. These findings indicate that the antibody used in this study is specific toward GHS-R1a. Moreover, we further determined whether ghrelin receptor was expressed in adult rat hippocampal NSCs using GHS-R1a antibody from a different company (Phoenix Pharmaceuticals, Burlingame, CA, USA) and found clear GHS-R1a immunoreactivity (data not shown). Collectively, these findings indicate that GHS-R1a protein is expressed in cultured adult rat hippocampal NSCs. The current results obtained from GHS-R1a antagonist experiments also support the existence of ghrelin receptor in these cells, where the effects of ghrelin on cell proliferation and phosphorylation were blocked when the cells were pretreated with the ghrelin receptor antagonist.

It has been reported that ghrelin can activate the PI3K/Akt and ERK1/2 pathways in neuronal cells and these pathways mediate the protective effect of ghrelin against ischemic injury (Chung *et al*. 2007, 2008). We have shown in this study that ghrelin strongly induces the activation of ERK1/2 and Akt, which are believed to play important roles in regulating the proliferation of neural progenitor cells (Weeber & Sweatt 2002, Hao *et al*. 2004, Peltier *et al*. 2007). Chemical inhibition of MEK/ERK1/2 and PI3K/Akt resulted in complete suppression of the proliferative effect of ghrelin, indicating that this peptide stimulated the proliferation of adult rat hippocampal NSCs through the activation of MEK/ERK1/2 and PI3K/Akt. These results are consistent with the previous reports that the dominant negative Akt decreases cell proliferation (Peltier *et al*. 2007) and that the inhibition of ERK blocks the proliferation of neural progenitor cells (Learish *et al*. 2000, Zhou *et al*. 2004).

Akt can phosphorylate its downstream effector proteins, such as GSK-3β (GSK3B), which is known to negatively regulate Wnt/β-catenin signaling (Song *et al*. 2005). In the current study, we showed that the phosphorylation of GSK-3β is increased by ghrelin. It has been reported that ghrelin increases the nuclear translocation of β-catenin (Chung *et al*. 2008). In that inhibitor of GSK-3β promotes the proliferation of progenitor cells while overexpression of β-catenin increases the proliferation (Adachi *et al*. 2007), our data suggest that PI3K/Akt-mediated inactivation of GSK-3β is at least partly responsible for the proliferative effect of ghrelin. In addition, the observation that ghrelin increased the phosphorylation of mTOR and $p70^{S6K}$ and inhibition of mTOR by rapamycin reversed ghrelin-induced increase in the proliferation of adult rat hippocampal NSCs suggests a potential role of mTOR/$p70^{S6K}$ signaling. Studies have demonstrated that mTOR/$p70^{S6K}$ is involved in neuronal proliferation, differentiation, and survival. The activation of mTOR/$p70^{S6K}$ signaling has been reported to increase the proliferation of NSCs *in vitro* (Ryu *et al*. 2003). Insulin-induced differentiation of neural progenitors and DHEA-mediated survival of hippocampal newborn neurons were attenuated by rapamycin (Han *et al*. 2008, Li *et al*. 2010). These findings support that mTOR/$p70^{S6K}$ signaling stimulates the proliferation of adult rat hippocampal NSCs.

Furthermore, in this study, we observed that ghrelin rapidly increased STAT3 phosphorylation and ghrelin-induced adult rat hippocampal NSC proliferation was blocked by the STAT3 inhibitor cucurbitacin I. Similar to our results, Garza *et al*. (2008) also found that leptin stimulates the proliferation of adult hippocampal neural progenitor cells through a mechanism that is dependent on the activation of STAT3. Although these findings suggest that the Jak2/STAT3 signaling pathway may have important roles in mediating the proliferative response of

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

Journal of Endocrinology

ghrelin in hippocampal NSCs, the role of STAT3 in the proliferation of progenitor cells seems complicated. In fact, it has been reported that the suppression of STAT3 promotes neurogenesis in cultured NSCs (Gu *et al*. 2005).

Considering that neurogenesis in the DG has been proposed to mediate hippocampus-dependent learning and memory (Zhao *et al*. 2008), ghrelin-induced stimulation of neurogenesis in hippocampal progenitor cells may play an important role in improving the ability of ghrelin to enhance memory performance. Indeed, ghrelin knockout mice showed impaired memory performance, which was reversed by the peripheral administration of ghrelin (Diano *et al*. 2006, Li *et al*. 2013). It has also been reported that enhanced memory performance in the hippocampus-dependent behavioral test (Fontan-Lozano *et al*. 2007, Adams *et al*. 2008) and increased hippocampal neurogenesis (Lee *et al*. 2002) are observed in calorie-restricted adult animals, where circulating ghrelin levels are increased (Lutter *et al*. 2008). However, in ischemic injury and epilepsy, memory impairments are caused by neuronal cell loss in the hippocampus (Squire & Zola 1996, Butler & Zeman 2008). Therefore, ghrelin-induced stimulation of hippocampal neurogenesis may play an important role in improving cognitive function.

In conclusion, we have demonstrated that ghrelin increases the cellular proliferation of adult rat hippocampal NSCs via the activation of its receptors. We have also shown that ghrelin strongly activates ERK1/2 and Akt and that the proliferative effects of this peptide are mediated by the MEK/ERK1/2 and PI3K/Akt pathways. In addition, we provide evidence that increased Akt signaling by ghrelin is associated with the downstream attenuation of GSK-3β and the activation of mTOR/p70^{S6K}. The analysis of signaling pathways also suggests that the activation of STAT3 is associated with the proliferative effect of ghrelin. Taken together, the data suggest that multiple signaling pathways, such as MEK/ERK1/2, PI3K/Akt/GSK-3β, PI3K/Akt/mTOR/p70^{S6K}, and Jak2/STAT3, are involved in ghrelin-induced proliferation of adult rat hippocampal NSCs. It remains to be determined whether the same signaling pathways operate *in vivo*.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (No. 20120009383).

Author contribution statement

H C designed the study, carried out the laboratory experiments, and collected the data. E L, Y K, and S K carried out the laboratory experiments. S P designed the study, analyzed and interpreted the data, and wrote the manuscript.

Acknowledgements

We extend our thanks to Dr Rhonda D Kineman (University of Illinois at Chicago, Chicago, IL, USA) for proofreading and editing this manuscript.

References

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J & Eriksson PS 2000 Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *Journal of Neuroscience* **20** 2896–2903.

Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschop MH *et al*. 2006 Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. *Journal of Clinical Investigation* **116** 3229–3239. (doi:10.1172/JCI29867)

Adachi K, Mirzadeh Z, Sakaguchi M, Yamashita T, Nikolcheva T, Gotoh Y, Peltz G, Gong L, Kawase T, Alvarez-Buylla A *et al*. 2007 β-Catenin signaling promotes proliferation of progenitor cells in the adult mouse subventricular zone. *Stem Cells* **25** 2827–2836. (doi:10.1634/stemcells.2007-0177)

Adams MM, Shi L, Linville MC, Forbes ME, Long AB, Bennett C, Newton IG, Carter CS, Sonntag WE, Riddle DR *et al*. 2008 Caloric restriction and age affect synaptic proteins in hippocampal CA3 and spatial learning ability. *Experimental Neurology* **211** 141–149. (doi:10.1016/j.expneurol.2008.01.016)

Andrews ZB 2011 The extra-hypothalamic actions of ghrelin on neuronal function. *Trends in Neurosciences* **34** 31–40. (doi:10.1016/j.tins.2010.10.001)

Baldanzi G, Filigheddu N, Cutrupi S, Catapano F, Bonissoni S, Fubini A, Malan D, Baj G, Granata R, Broglio F *et al*. 2002 Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. *Journal of Cell Biology* **159** 1029–1037. (doi:10.1083/jcb.200207165)

Brewer GJ, Torricelli JR, Evege EK & Price PJ 1993 Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *Journal of Neuroscience Research* **35** 567–576. (doi:10.1002/jnr.490350513)

Butler CR & Zeman AZ 2008 Recent insights into the impairment of memory in epilepsy: transient epileptic amnesia, accelerated long-term forgetting and remote memory impairment. *Brain* **131** 2243–2263. (doi:10.1093/brain/awn127)

Carlini VP, Varas MM, Cragnolini AB, Schioth HB, Scimonelli TN & de Barioglio SR 2004 Differential role of the hippocampus, amygdala, and dorsal raphe nucleus in regulating feeding, memory, and anxiety-like behavioral responses to ghrelin. *Biochemical and Biophysical Research Communications* **313** 635–641. (doi:10.1016/j.bbrc.2003.11.150)

Chung H, Kim E, Lee DH, Seo S, Ju S, Lee D, Kim H & Park S 2007 Ghrelin inhibits apoptosis in hypothalamic neuronal cells during oxygen-glucose deprivation. *Endocrinology* **148** 148–159. (doi:10.1210/en.2006-0991)

Chung H, Seo S, Moon M & Park S 2008 Phosphatidylinositol-3-kinase/Akt/glycogen synthase kinase-3β and ERK1/2 pathways mediate protective effects of acylated and unacylated ghrelin against oxygen-glucose deprivation-induced apoptosis in primary rat cortical neuronal cells. *Journal of Endocrinology* **198** 511–521. (doi:10.1677/JOE-08-0160)

Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K & Nakazato M 2000 Ghrelin, a novel growth

© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.
Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* **141** 4255–4261. (doi:10.1210/en.141.11.4255)

Delhanty PJ, van der Eerden BC, van der Velde M, Gauna C, Pols HA, Jahr H, Chiba H, van der Lely AJ & van Leeuwen JP 2006 Ghrelin and unacylated ghrelin stimulate human osteoblast growth via mitogen-activated protein kinase (MAPK)/phosphoinositide 3-kinase (PI3K) pathways in the absence of GHS-R1a. *Journal of Endocrinology* **188** 37–47. (doi:10.1677/joe.1.06404)

Diano S, Farr SA, Benoit SC, McNay EC, da Silva I, Horvath B, Gaskin FS, Nonaka N, Jaeger LB, Banks WA *et al.* 2006 Ghrelin controls hippocampal spine synapse density and memory performance. *Nature Neuroscience* **9** 381–388. (doi:10.1038/nn1656)

Fontan-Lozano A, Saez-Cassanelli JL, Inda MC, de los Santos-Arteaga M, Sierra-Dominguez SA, Lopez-Lluch G, Delgado-Garcia JM & Carrion AM 2007 Caloric restriction increases learning consolidation and facilitates synaptic plasticity through mechanisms dependent on NR2B subunits of the NMDA receptor. *Journal of Neuroscience* **27** 10185–10195. (doi:10.1523/JNEUROSCI.2757-07.2007)

Garza JC, Guo M, Zhang W & Lu XY 2008 Leptin increases adult hippocampal neurogenesis *in vivo* and *in vitro*. *Journal of Biological Chemistry* **283** 18238–18247. (doi:10.1074/jbc.M800053200)

Ghigo E, Broglio F, Arvat E, Maccario M, Papotti M & Muccioli G 2005 Ghrelin: more than a natural GH secretagogue and/or an orexigenic factor. *Clinical Endocrinology* **62** 1–17. (doi:10.1111/j.1365-2265.2004.02160.x)

Grote HE & Hannan AJ 2007 Regulators of adult neurogenesis in the healthy and diseased brain. *Clinical and Experimental Pharmacology & Physiology* **34** 533–545. (doi:10.1111/j.1440-1681.2007.04610.x)

Gu F, Hata R, Ma YJ, Tanaka J, Mitsuda N, Kumon Y, Hanakawa Y, Hashimoto K, Nakajima K & Sakanaka M 2005 Suppression of Stat3 promotes neurogenesis in cultured neural stem cells. *Journal of Neuroscience Research* **81** 163–171. (doi:10.1002/jnr.20561)

Guan XM, Yu H, Palyha OC, McKee KK, Feighner SD, Sirinathsinghji DJ, Smith RG, van der Ploeg LH & Howard AD 1997 Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. *Brain Research. Molecular Brain Research* **48** 23–29. (doi:10.1016/S0169-328X(97)00071-5)

Han J, Wang B, Xiao Z, Gao Y, Zhao Y, Zhang J, Chen B, Wang X & Dai J 2008 Mammalian target of rapamycin (mTOR) is involved in the neuronal differentiation of neural progenitors induced by insulin. *Molecular and Cellular Neuroscience* **39** 118–124. (doi:10.1016/j.mcn.2008.06.003)

Hao Y, Creson T, Zhang L, Li P, Du F, Yuan P, Gould TD, Manji HK & Chen G 2004 Mood stabilizer valproate promotes ERK pathway-dependent cortical neuronal growth and neurogenesis. *Journal of Neuroscience* **24** 6590–6599. (doi:10.1523/JNEUROSCI.5747-03.2004)

Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J *et al.* 1996 A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* **273** 974–977. (doi:10.1126/science.273.5277.974)

Hwang S, Moon M, Kim S, Hwang L, Ahn KJ & Park S 2009 Neuroprotective effect of ghrelin is associated with decreased expression of prostate apoptosis response-4. *Endocrine Journal* **56** 609–617. (doi:10.1507/endocrj.K09E-072)

Jiang H, Betancourt L & Smith RG 2006 Ghrelin amplifies dopamine signaling by cross talk involving formation of growth hormone secretagogue receptor/dopamine receptor subtype 1 heterodimers. *Molecular Endocrinology* **20** 1772–1785. (doi:10.1210/me.2005-0084)

Jiang H, Li LJ, Wang J & Xie JX 2008 Ghrelin antagonizes MPTP-induced neurotoxicity to the dopaminergic neurons in mouse substantia nigra. *Experimental Neurology* **212** 532–537. (doi:10.1016/j.expneurol.2008.05.006)

Johansson I, Destefanis S, Aberg ND, Aberg MA, Blomgren K, Zhu C, Ghe C, Granata R, Ghigo E, Muccioli G *et al.* 2008 Proliferative and protective effects of growth hormone secretagogues on adult rat hippocampal progenitor cells. *Endocrinology* **149** 2191–2199. (doi:10.1210/en.2007-0733)

Kojima M & Kangawa K 2005 Ghrelin: structure and function. *Physiological Reviews* **85** 495–522. (doi:10.1152/physrev.00012.2004)

Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H & Kangawa K 1999 Ghrelin is a growth hormone-releasing acylated peptide from stomach. *Nature* **402** 656–660. (doi:10.1038/45230)

Learish RD, Bruss MD & Haak-Frendscho M 2000 Inhibition of mitogen-activated protein kinase kinase blocks proliferation of neural progenitor cells. *Brain Research. Developmental Brain Research* **122** 97–109. (doi:10.1016/S0165-3806(00)00064-X)

Lee J, Seroogy KB & Mattson MP 2002 Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice. *Journal of Neurochemistry* **80** 539–547. (doi:10.1046/j.0022-3042.2001.00747.x)

Lee J, Lim E, Kim Y, Li E & Park S 2010*a* Ghrelin attenuates kainic acid-induced neuronal cell death in the mouse hippocampus. *Journal of Endocrinology* **205** 263–270. (doi:10.1677/JOE-10-0040)

Lee JY, Chung H, Yoo YS, Oh YJ, Oh TH, Park S & Yune TY 2010*b* Inhibition of apoptotic cell death by ghrelin improves functional recovery after spinal cord injury. *Endocrinology* **151** 3815–3826. (doi:10.1210/en.2009-1416)

Li L, Xu B, Zhu Y, Chen L, Sokabe M & Chen L 2010 DHEA prevents Aβ25-35-impaired survival of newborn neurons in the dentate gyrus through a modulation of PI3K–Akt–mTOR signaling. *Neuropharmacology* **59** 323–333. (doi:10.1016/j.neuropharm.2010.02.009)

Li E, Chung H, Kim Y, Kim DH, Ryu JH, Sato T, Kojima M & Park S 2013 Ghrelin directly stimulates adult hippocampal neurogenesis: implications for learning and memory. *Endocrine Journal*. In press (doi:10.1507/endocrj.EJ13-0008)

Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, Birnbaum S, Yanagisawa M, Elmquist JK, Nestler EJ *et al.* 2008 The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. *Nature Neuroscience* **11** 752–753. (doi:10.1038/nn.2139)

Miao Y, Xia Q, Hou Z, Zheng Y, Pan H & Zhu S 2007 Ghrelin protects cortical neuron against focal ischemia/reperfusion in rats. *Biochemical and Biophysical Research Communications* **359** 795–800. (doi:10.1016/j.bbrc.2007.05.192)

Ming GL & Song H 2005 Adult neurogenesis in the mammalian central nervous system. *Annual Review of Neuroscience* **28** 223–250. (doi:10.1146/annurev.neuro.28.051804.101459)

Moon M, Kim HG, Hwang L, Seo JH, Kim S, Hwang S, Kim S, Lee D, Chung H, Oh MS *et al.* 2009*a* Neuroprotective effect of ghrelin in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease by blocking microglial activation. *Neurotoxicity Research* **15** 332–347. (doi:10.1007/s12640-009-9037-x)

Moon M, Kim S, Hwang L & Park S 2009*b* Ghrelin regulates hippocampal neurogenesis in adult mice. *Endocrine Journal* **56** 525–531. (doi:10.1507/endocrj.K09E-089)

Naleid AM, Grace MK, Cummings DE & Levine AS 2005 Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. *Peptides* **26** 2274–2279. (doi:10.1016/j.peptides.2005.04.025)

Nogueiras R, Tovar S, Mitchell SE, Rayner DV, Archer ZA, Dieguez C & Williams LM 2004 Regulation of growth hormone secretagogue receptor gene expression in the arcuate nuclei of the rat by leptin and ghrelin. *Diabetes* **53** 2552–2558. (doi:10.2337/diabetes.53.10.2552)

Park YJ, Lee YJ, Kim SH, Joung DS, Kim BJ, So I, Park DJ & Cho BY 2008 Ghrelin enhances the proliferating effect of thyroid stimulating hormone in FRTL-5 thyroid cells. *Molecular and Cellular Endocrinology* **285** 19–25. (doi:10.1016/j.mce.2008.01.003)

Peltier J, O'Neill A & Schaffer DV 2007 PI3K/Akt and CREB regulate adult neural hippocampal progenitor proliferation and differentiation. *Developmental Neurobiology* **67** 1348–1361. (doi:10.1002/dneu.20506)

Ryu JK, Choi HB, Hatori K, Heisel RL, Pelech SL, McLarnon JG & Kim SU 2003 Adenosine triphosphate induces proliferation of human neural

http://joe.endocrinology-journals.org
DOI: 10.1530/JOE-13-0045
© 2013 Society for Endocrinology
Printed in Great Britain
Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access

stem cells: role of calcium and p70 ribosomal protein S6 kinase. *Journal of Neuroscience Research* **72** 352–362. (doi:10.1002/jnr.10507)

Sato M, Nakahara K, Goto S, Kaiya H, Miyazato M, Date Y, Nakazato M, Kangawa K & Murakami N 2006 Effects of ghrelin and des-acyl ghrelin on neurogenesis of the rat fetal spinal cord. *Biochemical and Biophysical Research Communications* **350** 598–603. (doi:10.1016/j.bbrc.2006.09.088)

Song G, Ouyang G & Bao S 2005 The activation of Akt/PKB signaling pathway and cell survival. *Journal of Cellular and Molecular Medicine* **9** 59–71. (doi:10.1111/j.1582-4934.2005.tb00337.x)

Squire LR & Zola SM 1996 Ischemic brain damage and memory impairment: a commentary. *Hippocampus* **6** 546–552. (doi:10.1002/(SICI)1098-1063(1996)6:5<546::AID-HIPO7>3.0.CO;2-G)

Thielemans L, Peeters PJ, Jonckheere H, Luyten W, de Hooqt R, Coulie B & Aerssens J 2007 The hepatocarcinoma cell line HepG2 does not express a GHS-R1a-type ghrelin receptor. *Journal of Receptor and Signal Transduction Research* **27** 309–322. (doi:10.1080/10799890701519587)

Van der Lely AJ, Tschop M, Heiman ML & Ghigo E 2004 Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocrine Reviews* **25** 426–457. (doi:10.1210/er.2002-0029)

Weeber EJ & Sweatt JD 2002 Molecular neurobiology of human cognition. *Neuron* **33** 845–848. (doi:10.1016/S0896-6273(02)00634-7)

Yoshimatsu T, Kawaguchi D, Oishi K, Takeda K, Akira S, Masuyama N & Gotoh Y 2006 Non-cell-autonomous action of STAT3 in maintenance of neural precursor cells in the mouse neocortex. *Development* **133** 2553–2563. (doi:10.1242/dev.02419)

Zhang W, Lin TR, Hu Y, Fan Y, Zhao L, Stuenkel EL & Mulholland MW 2004 Ghrelin stimulates neurogenesis in the dorsal motor nucleus of the vagus. *Journal of Physiology* **559** 729–737. (doi:10.1113/jphysiol.2004.064121)

Zhang W, Hu Y, Lin TR, Fan Y & Mulholland MW 2005 Stimulation of neurogenesis in rat nucleus of the solitary tract by ghrelin. *Peptides* **26** 2280–2288. (doi:10.1016/j.peptides.2005.04.023)

Zhao C, Deng W & Gage FH 2008 Mechanisms and functional implications of adult neurogenesis. *Cell* **132** 645–660. (doi:10.1016/j.cell.2008.01.033)

Zhou L, Del Villar K, Dong Z & Miller CA 2004 Neurogenesis response to hypoxia-induced cell death: map kinase signal transduction mechanisms. *Brain Research* **1021** 8–19. (doi:10.1016/j.brainres.2004.05.115)

Received in final form 16 April 2013
Accepted 22 April 2013
Accepted Preprint published online 22 April 2013

© 2013 Society for Endocrinology
Printed in Great Britain

Published by Bioscientifica Ltd.

Downloaded from Bioscientifica.com at 04/29/2025 05:22:16PM
via free access