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Leptin reverses corticosterone-induced inhibition of neural stem cell proliferation through activating the NR2B subunits of NMDA receptors

Wen-Zhu Shi [a,f,1], Yu-Liang Miao [b,1], Wen-Zhi Guo [c], Wei Wu [d,*], Bao-Wei Li [d,1], Li-Na An [e], Wei-Wu Fang [b], Wei-Dong Mi [f,*]

[a] Anesthesia and Operation Center, Hainan Branch of Chinese PLA General Hospital, Hainan 572013, China
[b] Department of Anesthesiology, PLA No. 306 Hospital, Beijing 100101, China
[c] Department of Anesthesiology, Beijing Military General Hospital of Chinese People's Liberation Army, Beijing 100700, China
[d] Department of Head and Neck Surgery of Otolaryngology, PLA No. 306 Hospital, Beijing 100101, China
[e] Department of Anesthesiology, Armed Police General Hospital, Beijing 100039, China
[f] Anesthesia and Operation Center, Chinese PLA General Hospital, Beijing 100853, China

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ABSTRACT

Corticosterone inhibits the proliferation of hippocampal neural stem cells (NSCs). The removal of corticosterone-induced inhibition of NSCs proliferation has been reported to contribute to neural regeneration. Leptin has been shown to regulate brain development, improve angiogenesis, and promote neural regeneration; however, its effects on corticosterone-induced inhibition of NSCs proliferation remain unclear. Here we reported that leptin significantly promoted the proliferation of hippocampal NSCs in a concentration-dependent pattern. Also, leptin efficiently reversed the inhibition of NSCs proliferation induced by corticosterone. Interestingly, pre-treatment with non-specific NMDA antagonist MK-801, specific NR2B antagonist Ro 25-6981, or small interfering RNA (siRNA) targeting NR2B, significantly blocked the effect of leptin on corticosterone-induced inhibition of NSCs proliferation. Furthermore, corticosterone significantly reduced the protein expression of NR2B, whereas pre-treatment with leptin greatly reversed the attenuation of NR2B expression caused by corticosterone in cultured hippocampal NSCs. Our findings demonstrate that leptin reverses the corticosterone-induced inhibition of NSCs proliferation. This process is, at least partially mediated by increased expression of NR2B subunits of NMDA receptors.

1. Introduction

Elevated gluococorticoids have been found in several pathophysiological conditions, including acute or chronic stress and depression, which may impair neural regeneration [1]. Corticosterone is a glucocorticoid secreted by the adrenal cortex, and can inhibit the proliferation and differentiation of NSCs through various pathways [2]. As glucocorticoid receptors are enriched in the hippocampus, increased corticosterone level suppresses neural regeneration and differentiation in the hippocampal region [3,4]. Hippocampal NSCs are involved in neural regeneration following multiple disorders or injuries [5]. Hence, attenuating the effects of NSCs exposure to corticosterone may promote neural regeneration and treatments of related diseases [6].

Leptin is a 16-kDa protein mainly expressed in adipose tissues. The leptin receptor (LepR) is widely expressed in the central nervous system and peripheral tissues. It is highly expressed in the hypothalamus as well as brain regions related to learning and memory, including the hippocampus and cortex. Accumulating evidence suggests that leptin acts as a crucial signal for brain development during both pre- and post-natal life by regulating neurogenesis, axonal growth, and synaptogenesis. Leptin was also found to induce neurogenesis in the central nervous system following stroke by activating the LepR. Nevertheless, it remains unclear whether or not leptin may promote the proliferation of NSCs derived from embryonic mouse hippocampus and relieave the corticosterone-mediated inhibition of hippocampal NSCs proliferation.

Leptin also indirectly activates the N-methyl-D-aspartate (NMDA) receptors (NMDARs) [7,8], which are essential in

* Corresponding authors. Address: Department of Head and Neck Surgery of Otolaryngology, PLA No. 306 Hospital, No. 9 Anxiangbeilu Road, Beijing 100101, China (W.Wu). Address: Anesthesia and Operation Center, Chinese PLA General Hospital, No. 28 Fuxing Road, Beijing 100853, China. Fax: +86 10 66938151 (W.D. Mi).
E-mail addresses: wwzwgk@163.com (W. Wu), elite2005gg@163.com (W.-D. Mi).
[1] These authors contributed equally to this work.

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modulating the proliferation of NSCs during the embryonic stage [9]. Among all NR2 subunits of the NMDARs, NR2B is the dominant subunit expressed in newborn nerve cells [10]. Recent studies have revealed that NR2B-containing NMDARs promote the neural stem/progenitor cell proliferation through the calcium/calmodulin-dependent protein kinase IV (CaMKIV) and cAMP response element binding protein (CREB) pathway [11]. Moreover, NR2B activation can enhance NSCs proliferation in the subventricular zone of neonatal rats following hypoxic–ischemic brain damage [12]. Therefore, we hypothesized that leptin may promote NSCs proliferation and mediate the inhibitory effects of corticosterone through activation of the NR2B subunit.

In this present study, we investigated the effects of leptin on the proliferation of the NSCs and its role in the inhibitory effects of corticosterone *in vitro*. Furthermore, we explored the potential mechanisms underlying this process.

2. Materials and methods

2.1. Reagents

Dulbecco's Modified Eagle Medium (DMEM)/F12 media, B27 and N2 were obtained from Invitrogen™ (Los Angeles, California, USA). Epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and mouse recombinant leptin were bought from Peprotech (Rocky Hill, NJ, USA). Poly-L-lysine (PLL), trypsin-EDTA, corticosterone, MK-801, Ro 25-6981, pre-designed NR2B siRNA oligonucleotides (#NM_008171), scrambled siRNA, MISSION® siRNA transfection reagent and anti-GAPDH antibody were purchased from Sigma (St. Louis, MO, USA). Rabbit anti-NMDAR2B primary antibody was provided by Abcam (Cambridge, MA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody was purchased from Zhongshan Biotechnology Co. Ltd., Beijing, China.

2.2. Primary culture of NSCs

Primary NSCs were dissociated from embryonic CD-1 mice provided by Vital River Laboratories, Beijing, China. The animal experiments were approved by the Animal Ethical Committee of the General Hospital of the People's Liberation Army. All procedures were performed according to the Guidance Suggestions for the Care and Use of Laboratory Animals established by the Ministry of Science and Technology of the People's Republic of China. A total of six female pregnant CD-1 mice were used in this study. Primary NSCs were isolated from each mouse. In brief, the hippocampi of 14-day-old embryonic mouse brains were dissected and cut into pieces. Single cell suspension solutions were prepared. Live cells were assessed by Typan blue dye exclusion assay using Count star cytometer (RuiYu Biotechnology Co., Ltd. Shanghai, China). Cells were seeded into PLL-precoated culture flasks at a density of 2×10^5 cells/mL and were maintained in DMEM/F12 media containing 2% B27, 1% N2, 20 ng/mL EGF and 20 ng/mL bFGF. Cells were incubated in a 5% CO_2-humidified incubator (Heraeus, Germany) at 37 °C, and the NSCs at this stage represented passage 1 (P1). Half of the culture medium was changed every 3 days. Six or seven days following initial seeding, neural spheres were digested by 0.1% Trypsin-EDTA solution for 10 min at 37 °C. The single cell suspension solutions were reseeded into new culture flask at a density of 2×10^5 cells/mL, and represented P2 cells. P2 or P3 cells were used for the experiments.

2.3. Determination of cell proliferation

NSCs proliferation was determined using the CCK-8 assay according to the manufacture's instructions (Sigma, St. Louis, MO, USA). In brief, cells were seeded into PLL-precoated 96-well plate at a density of 5×10^4 cells/mL ($n = 6$). After different concentrations of drug treatment, 10 μL of CCK-8 solution was added into each well and cells were incubated at 37 °C for 1 h. The optical density (OD) at 450 nm was examined using EnVision multilabel reader (PerkinElmer, Inc. Turku, Finland) to assess cell proliferation capability.

2.4. Transfection of NR2B siRNA

The siRNA oligonucleotides were diluted and transfected with MISSION® siRNA transfection reagent according to the manufacturer's instructions. Briefly, NSCs were seeded into Poly-L-Lysine-precoated 24-well plate at a density of 5×10^4 cells/mL and incubated in a 5% CO_2 incubator at 37 °C. Twenty four hours after initial seeding, cells were treated with a mixture of transfection reagent and 10, 20 or 40 nM siRNAs or non-specific siRNAs. After 6 h incubation, the mixture was replaced with culture medium, and cells were cultured for additional 48 h before harvesting for experiments.

2.5. Cellular ELISA

The expression of membrane NR2B was measured by cellular ELISA as described previously with minor modifications. Cells were fixed with 4% paraformaldehyde (PFA) for 60 min at room temperature. After being washed with phosphate buffered saline (PBS), cells were incubated with 100 mM glycine for 10 min. Next, cells were blocked with 5% calf serum for 1 h at 37 °C followed by incubation with rabbit anti-mouse NR2B primary antibody (1:200 dilution) at 4 °C overnight. After being washed with PBS, cells were probed with HRP-conjugated goat anti-rabbit IgG antibody (1:200 dilution) at 37 °C for 30 min. Cells were then treated with Streptavidin-Biotin Complex at room temperature for another 30 min. Plates were visualized by the addition of 20 μL/well of 4 mg/mL o-phenylenediamine (OPD) at room temperature for 20 min. The reaction was stopped by adding 150 μL/well HCl (3 mM). The absorbance of the orange–brown end product was measured at 490 nm (A_{490}), and was used to quantitate NR2B expression at the cellular membrane.

2.6. Western blot assay

The protein expression of NR2B in the cultured cells was also determined by Western blot assay. Total protein was extracted from cells using lysis buffer containing 1% Trition X-100, 1% deoxycholate and 0.1% SDS. Protein concentration was measured using a BCA protein assay (Pierce, Holmdel, NJ, USA). Equal amounts of protein extracts (40 μg) were then separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), probed with anti-NMDAR2B primary antibody (1:500 dilution, Abcam) or anti-GAPDH (1:2000 dilution, Sigma) primary antibody. The expression bands of target proteins were analyzed by Gelpro3.2 software (YuanAo International Trade Co. Ltd., HongKong). The densitometric values were used to conduct statistical analysis. The housekeeping protein GAPDH was used as an internal control.

2.7. Statistical analysis

Data were analyzed by GraphPadPrism (version 5.03, GraphPad Software Inc, CA, USA) software. All data were presented as mean ± standard error of the mean (SEM) of three independent experiments. Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple comparison post-hoc test. $P < 0.05$ was recognized as significantly different.

3. Results

3.1. Leptin promoted the proliferation of NSCs

In order to determine the effects of leptin on the proliferation of NSCs, we treated P2 or P3 cells with different concentrations (10–160 ng/mL) of leptin. After six days incubation, the cell proliferation capability was evaluated using a CCK-8 assay. NSC viability was evaluated using OD values. We found that 10–160 ng/mL leptin significantly promoted the proliferation of NSCs in a concentration-dependent pattern ($P < 0.01$ compared with control; Fig. 1). However, no significant difference in OD value was found among 40, 80 or 160 ng/mL leptin treatment groups ($P > 0.05$). Thus, 40 ng/mL leptin was used in the following experiments.

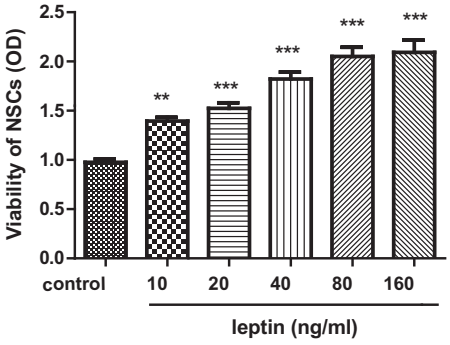

Fig. 1. Leptin promoted the proliferation of NSCs. Cells were incubated with different concentrations of leptin for six days. The proliferation capability was determined by a CCK-8 assay. Data are expressed as mean ± SEM from three independent experiments ($n = 6$). Statistical significance was determined by one-way ANOVA followed by post hoc Dunnett's multiple comparison test. $^{**}P < 0.01$, $^{***}P < 0.001$, vs. control group.

3.2. Effects of corticosterone, noncompetitive NMDAR antagonist MK-801, specific NR2B subunit antagonist Ro 25-6981 or NR2B siRNA on the proliferation of the NSCs

According to previous reports, corticosterone [13], MK-801 [9,11], or Ro 25-6981 [11] could inhibit the proliferation of NSCs, respectively. Moreover, we examined the inhibitory effect of NR2B siRNA on the proliferation of NSCs. Here NSCs were exposed to 1, 3 or 10 μM corticosterone; 1, 5 or 25 μM MK-801; 1, 3 or 10 μM Ro 25-6981; 10, 20 or 40 nM NR2B siRNA for 72 h, respectively. Using the CCK-8 assay, we observed that corticosterone, MK-801 or Ro 25-6981 concentration-dependently suppressed NSCs proliferation (Fig. 2A–C).

Meanwhile, 10, 20 and 40 nM of NR2B siRNA suppressed NSCs proliferation and non-specific NR2B siRNA didn't suppressed it (Fig. 2D). It was noted that 10 μM corticosterone, 25 μM MK-801, 10 μM Ro 25-6981 or 40 nM NR2B siRNA significantly inhibited cell proliferation, respectively ($P < 0.001$ compared with DMSO vehicle control). Therefore, the above concentrations of reagents were used for the following experiments.

3.3. MK-801, Ro 25-6981 and NR2B siRNA blocked the effects of leptin on corticosterone-inhibited cell proliferation

To investigate the potential mechanisms of leptin-mediated NSCs proliferation in the presence of corticosterone, cells were pre-treated with or without the MK-801 or Ro 25-6981 for 1 h, followed by the treatment with or without 40 ng/mL leptin for another 1 h. After that, cells were further incubated with 0.1% DMSO (vehicle control) with or without 10 μM corticosterone for 72 h. Meanwhile, non-specific siRNA and NR2B siRNA pre-treated cells were incubated with or without leptin for 1 h, then incubated with or without corticosterone for 72 h. We found that leptin reversed the corticosterone-inhibited NSCs proliferation, but this effect was blocked by MK-801, Ro 25-6981, or NR2B siRNA (Fig. 3A–C). Meanwhile, this effect wasn't be blocked by non-specific siRNA (data not shown). This indicates that leptin may suppress the

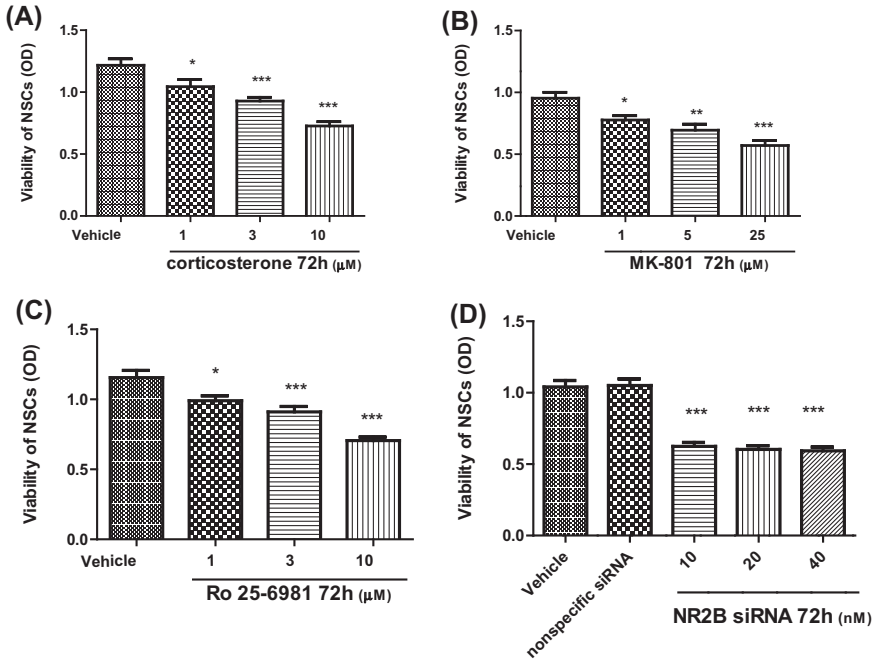

Fig. 2. Effects of corticosterone, MK-801, Ro 25-6981 or NR2B siRNA on inhibiting the proliferation of NSCs. Cells were incubated with 0.1% DMSO (vehicle control), 1, 3 or 10 μM corticosterone for 72 h (A); 1, 5 or 25 μM MK-801 for 72 h (B); 1, 5 or 25 μM Ro 25-6981 for 72 h (C); or transfected with non-specific siRNA or 10, 20 or 40 nM NR2B siRNA for 72 h (D). Data are expressed as mean ± SEM from three independent experiments ($n = 6$). Statistical significance was determined by one-way ANOVA followed by post hoc Dunnett's multiple comparison test. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, vs. vehicle group.

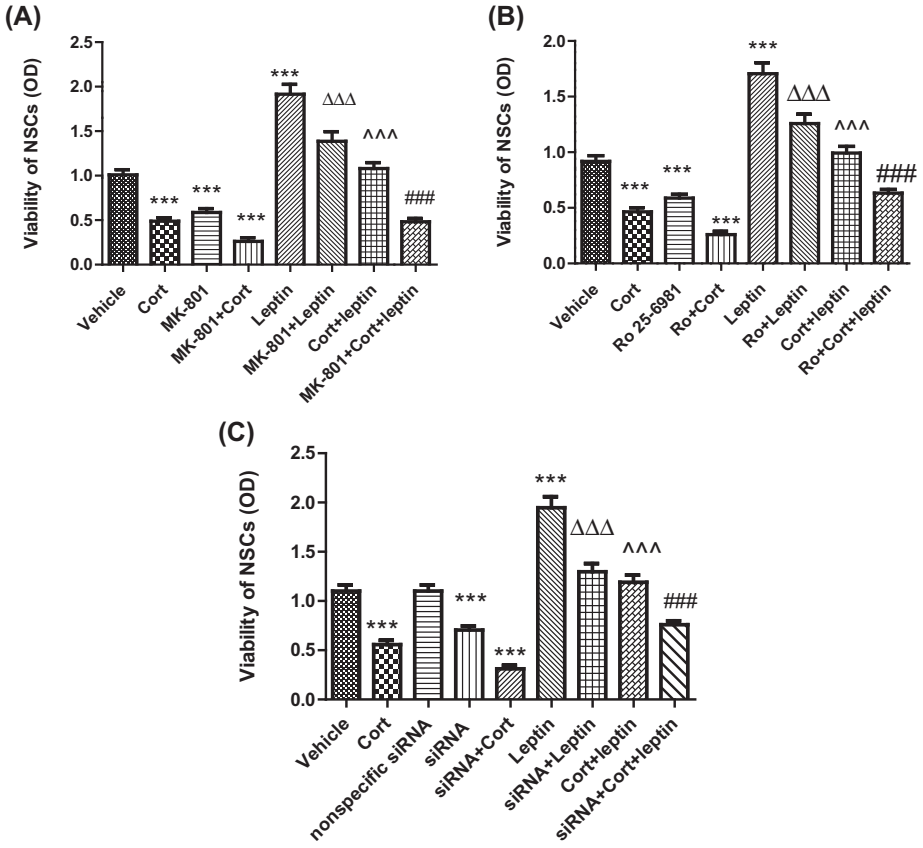

Fig. 3. Leptin suppressed the inhibitory role of corticosterone, whereas MK-801, Ro 25-6981 or NR2B siRNA blocked the effects of leptin on corticosterone-inhibited cell proliferation. Cells were pre-treated with or without 25 μM MK-801 (A) or 10 μM Ro 25-6981 (B) for 1 h followed by the treatment with or without 40 ng/mL leptin for another 1 h. After that, cells were further incubated with 0.1% DMSO (vehicle control), with or without 10 μM corticosterone for 72 h. (C) NR2B siRNA- or non-specific siRNA-transfected cells were treated with or without leptin for 1 h. After that, cells were further incubated with 0.1% DMSO (vehicle control) or with or without corticosterone for 72 h. The proliferation capability was determined by CCK-8 assay. Data were calculated from three independent experiments ($n = 6$). Statistical significance was determined by one-way ANOVA followed by post hoc Dunnett's multiple comparison test. $^{***}P < 0.001$, vs. vehicle group; $^{\wedge\wedge\wedge}P < 0.001$, vs. cort group, $^{\Delta\Delta\Delta}P < 0.001$, vs. MK-801 group, $^{\#\#\#}P < 0.001$, vs. cort + leptin group (A); $^{\wedge\wedge\wedge}P < 0.001$, vs. cort group, $^{\Delta\Delta\Delta}P < 0.001$, vs. Ro 25-6981 group, $^{\#\#\#}P < 0.001$, vs. cort + leptin group (B); $^{\wedge\wedge\wedge}P < 0.001$, vs. cort group, $^{\Delta\Delta\Delta}P < 0.001$, vs. siRNA group, $^{\#\#\#}P < 0.001$, vs. cort + leptin group (C).

inhibitory role of corticosterone on NSCs proliferation through activating the NMDAR and especially the NR2B pathway.

3.4. Leptin upregulated the expression of NR2B in corticosterone-treated NSCs

We next examined the expression of NR2B in NSCs following different treatments. As shown by cellular ELISA analysis results, 10 μM corticosterone and 40 nM NR2B siRNA significantly down-regulated the expression of NR2B on the membrane of NSCs (Fig. 4A, $P < 0.001$ compared with DMSO vehicle control). Furthermore, 40 ng/mL leptin alone increased the expression of NR2B in NSCs ($P < 0.001$ compared with DMSO vehicle control). Pre-treatment of leptin significantly elevated NR2B expression of corticosterone-exposed NSCs as compared to corticosterone exposure alone ($P < 0.001$). Pre-treatment of NR2B siRNA significantly decreased the expression of NR2B in cells pre-treated with leptin followed by corticosterone exposure, compared to pre-treated with leptin followed by corticosterone exposure ($P < 0.001$). Similar results were obtained when evaluating the expression of NR2B, as determined by Western blot assay (Fig. 4B).

4. Discussion

The physiological concentration of serum leptin is approximately 9.79 ± 1.73 ng/mL in human body, while its level increases to 50–100 ng/mL in obese patients [14]. The concentration of leptin commonly used for *in vitro* studies ranges from 10 to 100 ng/mL, however, there are no data available for the leptin level in the central nervous system of mammals or human [15,16]. In this present study, we demonstrated that leptin (10–160 ng/mL) promoted the proliferation of NSCs derived from embryonic mouse hippocampus in a concentration-dependent pattern. A leptin concentration of 40 ng/mL showed a significant stimulation of proliferation, whereas no statistical difference was found among 40, 80 and 160 ng/mL leptin treatment groups ($P > 0.05$). These results were in accordance with the previous report in which Desai et al. showed that 10, 20 or 40 ng/mL leptin promoted the proliferation of fetal hypothalamic NSCs derived from embryonic day 20 fetal rats [17]. In addition, accumulating evidence shows that leptin can increase the proliferation of human nucleus pulposus cells, chondrocytes, and human prostate cells. Although different cell types and different concentrations of leptin were used, similar results were obtained as those seen in the current study.

Corticosterone has been found to inhibit the proliferation and differentiation of NSCs [18,19]. In this study, we also observed that 10 μM corticosterone significantly reduced the proliferation of cultured NSCs after 72 h incubation. Importantly, we first found that 40 ng/mL leptin greatly reversed corticosterone-mediated inhibition of NSCs proliferation.

We next determined the underlying mechanism(s) of leptin-induced NSCs proliferation following treatment with corticosterone. NMDARs are involved in the neurogenesis, differentiation, migration and synaptic plasticity [20]. During embryonic brain

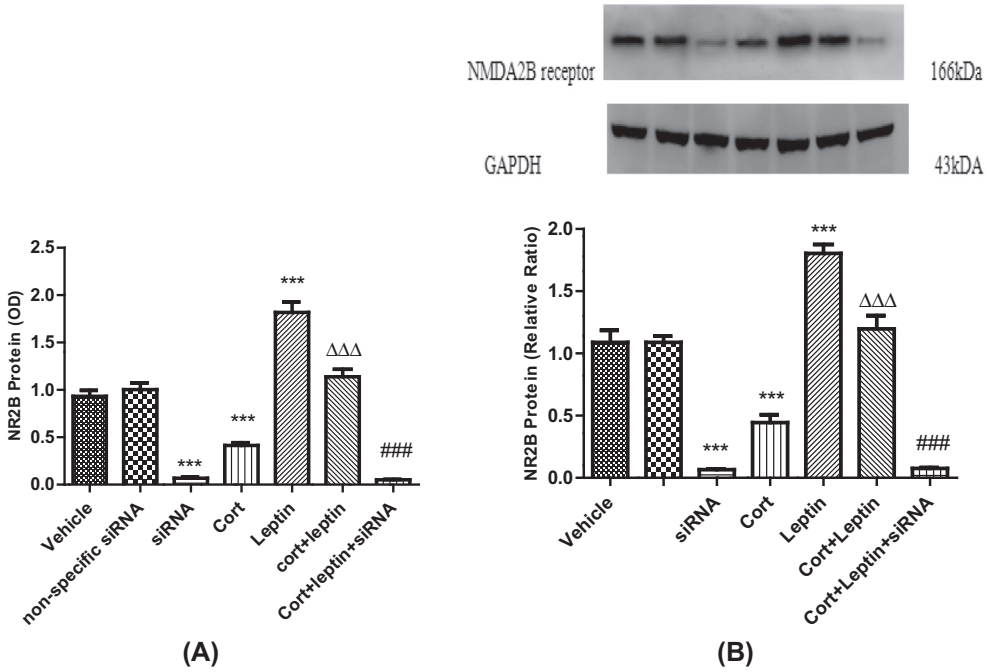

Fig. 4. Leptin upregulated the expression of NR2B in corticosterone-treated NSCs. NR2B siRNA- or non-specific siRNA-transfected cells were treated with or without 40 ng/mL leptin for 1 h followed by the incubation of 0.1% DMSO or with or without 10 μM corticosterone for another 72 h. The expression of NR2B in NSCs was examined by ELISA (A) or Western blot assay (B). Data were calculated from three independent experiments. Statistical significance was determined by one-way ANOVA followed by post hoc Dunnett's multiple comparison test. $^{***}P < 0.001$ vs. vehicle control; $^{\Delta\Delta\Delta}P < 0.001$ vs. the group treated with corticosterone alone; $^{\#\#\#}P < 0.001$ vs.cort + leptin group.

development, both the pharmacological inhibition and over-activation of NMDARs contribute to neural loss and cell apoptosis. Native NMDARs are assembled from NMDA receptor 1 (NR1) and NR2 subunits. Among them, NR2A and NR2B are the main subunits that regulate the activation of NMDARs and calcium ion influx [21]. It should be noted that NR2B are enriched in the neonatal neural cells [22]. Increased NR2B expression has been detected in proliferating NSCs induced by NMDAR activation [9,11]. Moreover, blockage of NR2B by the specific antagonist Ro 25-6981 remarkably inhibited NSCs proliferation and differentiation [11]. Son et al. [23]. and Huang et al. [24] demonstrated that in chronic immobilization stress-treated pregnant mice, serum corticosterone level was increased in pregnant mice, and the NR2B expression in synapses of the fetus hippocampus or in the hippocampus of offspring was decreased, leading to significantly impaired spatial learning and memory function of the offspring.

A variety of studies demonstrate that leptin may quickly activate NMDARs through various signal pathways, including janus kinase-signal transducer and activator of transcription (Jak-Stat) [8], phosphatidyl-inositol-3-kinase (PI3K) [25] and mitogen-activated protein kinase (MAPK) [26], and subsequently initiate proliferation-related gene transcription. It has been suggested that NR2B participates in hippocampus neuroregeneration, and that the downregulation of NR2B suppresses neuroregeneration [27]. Here we reported that leptin suppressed corticosterone-mediated inhibition of proliferation, and this effect could be blocked by the MK-801, Ro 25-6981 or NR2B siRNA. Cellular ELISA and western blot assay demonstrated that corticosterone significantly downregulated the expression of NR2B on the membrane of NSCs. Pre-treatment of leptin significantly elevated NR2B expression in the cells exposed to corticosterone. Pre-treatment of NR2B siRNA significantly decreased the expression of NR2B in the cells treated by leptin followed by corticosterone exposure. However, pre-treatment of non-specific NR2B siRNA didn't decrease it. These results indicated that NR2B was involved in the mechanism of the leptin-reversed inhibition of corticosterone-induced NSCs proliferation. Future studies will be conducted to clarify the potential up- and down-stream signals of NR2B subunits following leptin treatment. In addition, the activation of NMDARs has been reported to inhibit cell apoptosis, which further contributes to growth [28]. Thus, we can not exclude the possibility that the cytoprotective effect of leptin against corticosterone is due to inhibition of apoptosis.

In summary, our findings reveal that leptin promotes the proliferation of NSCs derived from embryonic day 14 CD-1 mouse hippocampus. Additionally, leptin efficiently suppresses the corticosterone-mediated inhibition of NSCs proliferation, at least partially through activating the NR2B subunits of NMDARs. Our data provide valuable insights into understanding the pharmacological effects of leptin on the proliferation of NSCs in the presence or absence of high concentrations of corticosterone.

Acknowledgments

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References

[1] C.K. Barha, S. Brummelte, S.E. Lieblich, L.A. Galea, Chronic restraint stress in adolescence differentially influences hypothalamic-pituitary-adrenal axis function and adult hippocampal neurogenesis in male and female rats, Hippocampus 21 (11) (2011) 1216–1227.
[2] A. Gulino, E. De Smaele, E. Ferretti, Glucocorticoids and neonatal brain injury: the hedgehog connection, J. Clin. Invest. 119 (2) (2009) 243–246.
[3] T. Yabe, H. Hirahara, N. Harada, N. Ito, T. Nagai, T. Sanagi, et al., Ferulic acid induces neural progenitor cell proliferation in vitro and in vivo, Neuroscience 165 (2) (2010) 515–524.
[4] S. Alboni, F. Tascedda, D. Corsini, C. Benatti, F. Caggia, G. Capone, et al., Stress induces altered CRE/CREB pathway activity and BDNF expression in the hippocampus of glucocorticoid receptor-impaired mice, Neuropharmacology 60 (7–8) (2011) 1337–1346.
[5] A.J. Cole, Functional repair of the hippocampus by neural progenitors, Epilepsy Curr. 2 (6) (2002) 203–204.
[6] C. Tronche, C. Pierard, M. Coutan, F. Chauveau, P. Liscia, D. Beracochea, Increased stress-induced intra-hippocampus corticosterone rise associated

with memory impairments in middle-aged mice, Neurobiol. Learn. Mem. 93 (3) (2010) 343–351.
[7] P.R. Moult, J. Harvey, NMDA receptor subunit composition determines the polarity of leptin-induced synaptic plasticity, Neuropharmacology 61 (5–6) (2011) 924–936.
[8] Y. Tian, S. Wang, Y. Ma, G. Lim, H. Kim, J. Mao, Leptin enhances NMDA-induced spinal excitation in rats: a functional link between adipocytokine and neuropathic pain, Pain 152 (6) (2011) 1263–1271.
[9] J.Y. Joo, B.W. Kim, J.S. Lee, J.Y. Park, S. Kim, Y.J. Yun, et al., Activation of NMDA receptors increases proliferation and differentiation of hippocampal neural progenitor cells, J. Cell Sci. 120 (Pt 8) (2007) 1358–1370.
[10] P. Paoletti, J. Neyton, NMDA receptor subunits: function and pharmacology, Curr. Opin. Pharmacol. 7 (1) (2007) 39–47.
[11] M. Li, D.Q. Zhang, X.Z. Wang, T.J. Xu, NR2B-containing NMDA receptors promote neural progenitor cell proliferation through CaMKIV/CREB pathway, Biochem. Biophys. Res. Commun. 411 (4) (2011) 667–672.
[12] G.Y. Cui, W. Zhang, X.C. Ye, Q.C. Zhao, X. Shen, Effect of N-methyl-D-aspartic acid receptor subunit 2B antagonist on the proliferation of neural stem cells in the subventricular zone of the hypoxic-ischemic brain damage neonatal rats, Chin. J. Clin. Neurosci. 20 (6) (2012) 614–618.
[13] M. Sundberg, S. Savola, A. Hienola, L. Korhonen, D. Lindholm, Glucocorticoid hormones decrease proliferation of embryonic neural stem cells through ubiquitin-mediated degradation of cyclin D1, J. Neurosci. 26 (20) (2006) 5402–5410.
[14] R.V. Considine, M.K. Sinha, M.L. Heiman, A. Kriauciunas, T.W. Stephens, M.R. Nyce, et al., Serum immunoreactive-leptin concentrations in normal-weight and obese humans, N. Engl. J. Med. 334 (5) (1996) 292–295.
[15] A.M. Pino, J.M. Rodriguez, S. Rios, P. Astudillo, L. Leiva, G. Seitz, et al., Aromatase activity of human mesenchymal stem cells is stimulated by early differentiation, vitamin D and leptin, J. Endocrinol. 191 (3) (2006) 715–725.
[16] J. Udagawa, A. Ono, M. Kawamoto, H. Otani, Leptin and its intracellular signaling pathway maintains the neurosphere, Neuroreport 21 (18) (2010) 1140–1145.
[17] M. Desai, T. Li, M.G. Ross, Fetal hypothalamic neuroprogenitor cell culture: preferential differentiation paths induced by leptin and insulin, Endocrinology 152 (8) (2011) 3192–3201.
[18] R. Crupi, E. Mazzon, A. Marino, G. La Spada, P. Bramanti, S. Cuzzocrea, et al., Melatonin treatment mimics the antidepressant action in chronic corticosterone-treated mice, J. Pineal Res. 49 (2) (2010) 123–129.
[19] F. Murray, D.W. Smith, P.H. Hutson, Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice, Eur. J. Pharmacol. 583 (1) (2008) 115–127.
[20] K.A. Waters, R. Machaalani, NMDA receptors in the developing brain and effects of noxious insults, Neurosignals 13 (4) (2004) 162–174.
[21] D.R. Madden, The structure and function of glutamate receptor ion channels, Nat. Rev. Neurosci. 3 (2) (2002) 91–101.
[22] J. Nacher, E. Varea, J. Miguel Blasco-Ibanez, M.A. Gomez-Climent, E. Castillo-Gomez, C. Crespo, et al., N-methyl-d-aspartate receptor expression during adult neurogenesis in the rat dentate gyrus, Neuroscience 144 (3) (2007) 855–864.
[23] G.H. Son, D. Geum, S. Chung, E.J. Kim, J.H. Jo, C.M. Kim, et al., Maternal stress produces learning deficits associated with impairment of NMDA receptor-mediated synaptic plasticity, J. Neurosci. 26 (12) (2006) 3309–3318.
[24] Y. Huang, X. Shi, H. Xu, H. Yang, T. Chen, S. Chen, et al., Chronic unpredictable stress before pregnancy reduce the expression of brain-derived neurotrophic factor and N-methyl-D-aspartate receptor in hippocampus of offspring rats associated with impairment of memory, Neurochem. Res. 35 (7) (2010) 1038–1049.
[25] M. Durakoglugil, A.J. Irving, J. Harvey, Leptin induces a novel form of NMDA receptor-dependent long-term depression, J. Neurochem. 95 (2) (2005) 396–405.
[26] A.J. Irving, L. Wallace, D. Durakoglugil, J. Harvey, Leptin enhances NR2B-mediated N-methyl-D-aspartate responses via a mitogen-activated protein kinase-dependent process in cerebellar granule cells, Neuroscience 138 (4) (2006) 1137–1148.
[27] J. Ren, X. Li, X. Zhang, M. Li, Y. Wang, Y. Ma, The effects of intra-hippocampal microinfusion of D-cycloserine on fear extinction, and the expression of NMDA receptor subunit NR2B and neurogenesis in the hippocampus in rats, Prog. Neuropsychopharmacol. Biol. Psychiatry 44 (2013) 257–264.
[28] M. Hernandez, I. Guerrikagoitia, L. Martinez-Millan, E. Vecino, NMDA-receptor blockade enhances cell apoptosis in the developing retina of the postnatal rat, Int. J. Dev. Biol. 51 (2) (2007) 117–122.