J Pharmacol Sci **119**, 30 – 39 (2012)

Journal of Pharmacological Sciences

Full Paper

Glucocorticoids Decrease Astrocyte Numbers by Reducing Glucocorticoid Receptor Expression In Vitro and In Vivo

Kazuhiro Unemura[1], Toshiaki Kume[1], Minami Kondo[1], Yuki Maeda[1], Yasuhiko Izumi[1], and Akinori Akaike[1,*]

[1]*Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan*

Received February 20, 2012; Accepted March 12, 2012

Abstract. Glucocorticoids are stress hormones released from the adrenal cortex and their concentration is controlled by the hypothalamic–pituitary–adrenal axis. In this study, we investigated the effect of glucocorticoids on the number of astrocytes and glucocorticoid receptor (GR) expression in vitro and in vivo. Proliferation of cultured astrocytes was reduced following treatment with corticosterone and dexamethasone for 72 h. Corticosterone and dexamethasone also reduced GR expression in astrocytes. RU486, a GR antagonist, inhibited the reduction in both astrocyte proliferation and GR expression. Furthermore, GR knockdown by siRNA inhibited astrocyte proliferation. We also examined the effect of excessive glucocorticoid release on GR expression and the number of astrocytes in vivo by administering adrenocorticotropic hormone to rats for 14 days. GR expression was reduced in the prefrontal cortex and hippocampus and the number of astrocytes was reduced in the frontal cortex. Overall, our results suggest that glucocorticoids decrease the number of astrocytes by reducing GR expression.

Keywords: glucocorticoid, astrocyte, glucocorticoid receptor, adrenocorticotropic hormone (ACTH)

Introduction

Astrocytes are the most abundant cells in the central nervous system (CNS) and exist ubiquitously both in the developing and mature CNS. These cells are assumed to play an important role in the regulation of the brain environment. Astrocyte proliferation that occurs following brain injury, known as reactive gliosis, is well understood. However, astrocyte proliferation also occurs under normal physiological conditions. Glial cell numbers in the prefrontal cortex increase between adolescence and adulthood (1). Furthermore, exercise and learning enhances gliogenesis and astrocyte proliferation in the prefrontal cortex (2 – 4). Glial fibrillary acidic protein (GFAP)-expressing cells proliferate in the hippocampus and striatum of the adult brain under normal physiological conditions (5 – 7). Postmortem studies of tissue from young patients with major depressive disorder have previously demonstrated a reduced number of astrocytes in the prefrontal cortex (8). GFAP-positive cells have been shown to decrease in various regions including the prefrontal cortex in a rat model of depression as well as in clinical studies (9). Interestingly, it was reported that microinjection of L-α-aminoadipic acid, a gliotoxin, into the forebrain induced depression-like behaviors in rats (10). These studies suggest that astrocyte loss in the frontal cortex may be involved in the pathogenesis of depression.

Glucocorticoids, the hormonal end products of the hypothalamic–pituitary–adrenal axis (HPA axis), are known to increase with sustained stress. Released glucocorticoids activate a negative feedback system in the hippocampus, hypothalamus, and pituitary, which subsequently suppresses the release of glucocorticoids. However, in depression and stress disorders, glucocorticoids are hypersecreted because of dysfunction of this feedback system (11, 12). The glucocorticoid receptor (GR) plays an important role in the negative feedback system of the HPA axis. Some studies using GR-deficient mice have shown that GR deletion can induce hyperactivation of

*Corresponding author. aakaike@pharm.kyoto-u.ac.jp
Published online in J-STAGE on April 28, 2012 (in advance)
doi: 10.1254/jphs.12047FP

the HPA axis and lead to depression-like behaviors (13 – 15).

In view of the relationship between astrocytes and glucocorticoids, previous studies suggested that GR activation is involved in the inhibition of astrocyte proliferation (16, 17). However, the mechanisms by which this occurs remain unclear. In the present study, we examined the effect of glucocorticoids on astrocyte proliferation and GR expression. We also investigated whether excessive activation of the HPA axis affects astrocyte proliferation in vivo using a repeated adrenocorticotropic hormone (ACTH) administration rat model.

Materials and Methods

Materials

Eagle's minimum essential medium (MEM) was purchased from Nissui Pharmaceutical (Tokyo). Fetal bovine serum was obtained from JRH Biosciences (Lenexa, KS, USA). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT), glucose, glutamine, glutamate, HEPES, $NaHCO_3$, hydrochloric acid (HCl), trypsin, paraformaldehyde (PFA), and corticosterone were from Nacalai Tesque (Kyoto). RU486 and eplerenone were purchased from Sigma (St. Louis, MO, USA). Dispase was obtained from Gibco (Invitrogen Japan, Tokyo). Hydrogen peroxide (H_2O_2) and dexamethasone was obtained from Wako Chemicals (Osaka). 4′,6-Diamino-2-phenylindole (DAPI) was from Dojindo Laboratory (Kumamoto). ACTH-(1-24)-zinc (Cortrosyn-Z) was purchased from Daiichi Sankyo (Tokyo).

Animals

Experiments were carried out using postnatal day 1 pups of Wistar rats for primary cortical astrocyte monoculture and male Wistar rats weighing 180 – 220 g for repeated ACTH administration, which were purchased from Japan SLC (Shizuoka). All animal studies were conducted in accordance with the Ethical Guidance of the Kyoto University Animal Experimentation Committee and the Guidance of The Japanese Pharmacological Society. All procedures were approved by the Animal Research Committee, Graduate School of Pharmaceutical Science, Kyoto University.

Primary cortical astrocyte monoculture

Cortical type 1 astrocytes were prepared from postnatal day 1 pups of Wistar rats according to previously described procedures (18). In brief, the cells were dissociated from the cerebral cortices of neonates with dispase, filtered through a stainless mesh, and plated on uncoated 75-cm^2 flasks. Cultures were incubated in Eagle's MEM supplemented with 10% heat-inactivated fetal bovine serum, glutamine (2 mM), glucose (total 11 mM), $NaHCO_3$ (24 mM), and HEPES (10 mM) at 37°C in a humidified 5% CO_2 atmosphere. After the cells became confluent, non-astrocytes such as microglia were removed by shaking followed by changing the medium, and astrocytes were detached using trypsin and reseeded on 60-mm dishes or 24-well plates. The astrocytes were used for experiments 1 – 2 weeks after plating. The purity of astrocytes was > 95% as determined by immunostaining with anti-GFAP antibody. For immunoblotting, the cells were washed three times with cold Tris-buffered saline, harvested using a cell scraper, and centrifuged at 15,000 rpm at 4°C for 20 min. The precipitate was lysed with lysis buffer. For immunocytochemistry, the cells were fixed with 4% PFA for 10 min at room temperature and rinsed three times with phosphate-buffered saline (PBS).

Immunoblotting

Samples were lysed in buffer containing Tris (20 mM), β-glycerophosphate (25 mM), ethylene glycol tetraacetic acid (EGTA) (2 mM), Triton X-100 (1%), phenylmethylsulfonyl fluoride (1 mM), aprotinin (1%), dithiothreitol (2 mM), and vanadate (1 mM) on ice. Lysates were centrifuged at 15,000 rpm at 4°C for 30 min. The supernatants were mixed with a sample buffer containing 124 mM Tris-HCl (pH 6.8), 4% sodium dodecyl sulfate (SDS), 10% glycerol, 0.02% bromophenol blue, and 4% 2-mercaptoethanol. After denaturing by heating at 100°C for 5 min, an aliquot of the supernatant was loaded onto a SDS polyacrylamide gel, separated electrophoretically, and transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was then incubated in 10 mM Tris-buffered saline containing 0.1% Tween 20 and 5% dehydrated skim milk to block non-specific protein binding. The membrane was subsequently incubated with anti-GR antibody (1:200; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (1:100000; Ambion, Austin, TX, USA), or anti-GFAP antibody (1:1000; Calbiochem, San Diego, CA, USA). This was followed by incubation with secondary antibodies. Immunodetection was performed using the ECL Western Blotting detection system (Amersham Biosciences, Buckinghamshire, UK). The protein expression level was quantified using Image J software.

Immunochemistry

Brain samples and cultures were blocked with 1.5% or 5% goat serum and 0.3% Triton in PBS for 1 h. Subsequently, the samples were incubated with primary antibodies at 4°C overnight. Primary antibodies used were

anti-GFAP (1:1000; Dako, Glostrup, Denmark) antibody, anti-GR antibody (1:50), anti-neuronal nuclei (NeuN) antibody (1:50; Chemicon International, Temecula, CA, USA), and anti-bromodeoxyuridine (BrdU) antibody (1:500; Roche, Basel, Switzerland). After three washes with PBS, the samples were incubated for 90 min at room temperature with secondary antibodies, Alexa Fluor 488-labeled goat anti-mouse IgG or Alexa Fluor 594-labeled goat anti-rabbit IgG. Antibodies were diluted in PBS with goat serum (1% for in vitro and 1.5% for in vivo) and 0.3% Triton. Nuclei were stained with DAPI. Labeled cells were visualized and photographed using an epifluorescence microscope (BZ-8000; Keyence, Osaka). The number of cells in a randomly selected field (330 × 440 μm) was counted.

Repeated ACTH administration and sampling in vivo

Male Wistar rats weighing 180 – 210 g were used. The rats were maintained at a constant ambient temperature (22°C ± 1°C) under a 12-h light/dark cycle. We administered ACTH (100 μg/day, s.c.) to rats on a daily basis between 13:00 – 15:00 h. Under deep anesthesia (injection of pentobarbital, 100 mg/kg), the rats were sacrificed on day 15 following chronic ACTH administration. To obtain samples for immunoblotting, the rats were perfused transcardially with 60 ml cold PBS. Their brains were dissected into 1-mm coronal sections on a slicer, and the slices containing the frontal cortex were subsequently homogenized in an ice-cold lysis buffer. To obtain samples for immunohistochemistry, the rats were perfused transcardially with 60 ml cold PBS followed by 60 ml of 4% PFA. Their brains were isolated, fixed in 4% PFA for 2 h, and then soaked in 15% sucrose overnight at 4°C. After freezing, the brains were sliced into 16-μm-thick sections using a cryostat (Leica Microsystems, Wetzlar, Germany) and attached to silanized glass slides. Activation of NeuN and GFAP antigens was achieved by soaking the specimens in 10 mM citric acid (pH 6.0) for 15 min at 121°C in an autoclave. NeuN- and GFAP-positive cells were counted from six fields located between +4 and +5 mm from the bregma for each rat. GFAP-positive cells were counted if they had at least four distinct processes and were not associated with the formation of a blood vessel, according to the methods described previously (9).

MTT assay

To assess cell viability, cell metabolic activity was measured by an MTT assay. The cultures were incubated in serum-free medium containing 0.5 mg/ml of MTT tetrazolium salt for 2 h at 37°C. The medium was then aspirated and colored formazan products were solubilized in isopropanol. After agitation on a shaker, aliquots were transferred to a 96-well plate, and the absorbance was measured at 595 nm using a microplate reader (Bio-Rad Laboratories). MTT reduction activity and cell viability were expressed as a percentage of the absorbance measured in the control.

Lactate dehydrogenase (LDH) release assay

To estimate cytotoxicity, the amount of LDH released into the culture medium was measured using a MTX-LDH kit (Kyokuto Pharmaceutical Industrial Corp., Tokyo). In this assay, NAD is reduced to NADH through the conversion of lactate to pyruvate by LDH. NADH then reduces tetrazolium dyes to formazan dyes in the presence of diaphorase. In brief, 30 μl of culture supernatant was mixed with 70 μl of the LDH substrate mixture in a 96-well plate. After incubation for 30 min at 37°C, the reaction was stopped by adding 100 μl of 1 N HCl, and the absorbance was measured at 570 nm using a microplate reader. The background absorbance, calculated as the absorbance of the culture medium in the absence of cells, was subtracted from each absorbance value obtained.

Small interfering RNA (siRNA)

siRNAs were purchased from Nippon EGT (Toyama). The siRNA sequence targeting GR was 5′-UUACCUU UGUGCUGGAAGATT-3′. The sequence used for the scrambled control siRNA was 5′-GGACAUCGAGUA AAAGGGATT-3′. Cortical astrocytes were transfected with each siRNA using lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The medium was changed 6 h after transfection and cultures were incubated further.

Drug treatment

Primary astrocyte cell cultures were incubated in a medium containing corticosterone (0.01 – 1 μM), dexamethasone (0.01 – 1 μM), RU486 (0.3 – 3 μM), eplerenone (10 μM), or BrdU (1 μM).

Statistics

Values were expressed as the mean ± S.E.M. The statistical significance of difference between groups was determined using Tukey's test for the MTT, LDH, and BrdU assay, Dunnett's test for immunoblotting, and unpaired *t*-test for comparison between two groups. Probability values less than 5% were considered to be significant.

Results

GR was primarily expressed within the nuclei of cortical astrocytes

First, we examined the subcellular localization of GR in astrocytes in monoculture. Immunocytochemical analysis showed that GR was mainly expressed in the nuclei (Fig. 1). Even after treating the astrocytes with glucocorticoids, GRs were still primarily expressed in the nuclei (data not shown).

Glucocorticoids reduced astrocyte numbers via GR but did not induce astrocytic damage

We investigated the effects of glucocorticoids on the number of astrocytes in culture. Treatment with corticosterone (0.1 – 1 μM) and dexamethasone (0.01 – 1 μM) for 72 h significantly reduced the number of astrocytes, as determined in the MTT assay (Fig. 2: A, B). We investigated whether glucocorticoid treatment resulted in the death of astrocytes. Corticosterone (1 μM) and dexamethasone (1 μM) did not increase LDH release from astrocytes (Fig. 2C). However, H_2O_2, a positive control of cytotoxicity, did induce LDH release. Thus, these results suggest that glucocorticoid treatment reduced the number of astrocytes in vitro without causing cytotoxicity.

Glucocorticoids are known to interact with both GR and the mineralcorticoid receptor (MR). Both GR and MR are expressed in cultured astrocytes (19). We sought to determine which of these two receptors mediated the effects of glucocorticoids in the astrocytes. RU486 (3 μM), a GR antagonist, inhibited the effects of corticosterone and dexamethasone, whereas eplerenone (10 μM), an MR antagonist, did not (Fig. 2: D – F). These results suggest that glucocorticoids reduced the number of astrocytes via GR activation.

Glucocorticoids inhibited astrocyte proliferation via GR

We examined the effects of glucocorticoids on astrocyte proliferation by a BrdU incorporation assay. Corticosterone (1 μM) and dexamethasone (1 μM) reduced the number of BrdU-incorporated (BrdU-positive) astrocytes. RU486 (3 μM) significantly inhibited the glucocorticoid-induced reduction in BrdU-positive astrocytes (Fig. 3). These results suggest that glucocorticoids reduced the number of astrocytes by inhibiting astrocyte proliferation via GR.

Glucocorticoids altered GR expression in astrocyte culture

We subsequently investigated the effect of glucocorticoids on GR expression in astrocytes. Short-term (1 – 6 h) glucocorticoid treatment (1 μM) increased GR expression. In contrast, long-term (> 12 h) glucocorticoid treatment decreased GR expression (Fig. 4). RU486 (3 μM) transiently increased GR expression to a similar extent as that induced by glucocorticoids. However, long-term RU486 treatment did not decrease GR expression to levels below the control level (Fig. 4). These results suggest that glucocorticoids transiently increased GR expression in astrocytes with subsequent reduction in the expression.

RU486 inhibited downregulation but not upregulation of GR expression by glucocorticoids

We examined whether a GR antagonist could affect glucocorticoid-induced changes in GR expression in the astrocytes. First, we investigated the effect of RU486 on the increase in GR expression induced by short-term glucocorticoid treatment. Six hours of exposure to corticosterone (1 μM) and dexamethasone (1 μM) increased GR expression in the astrocytes. RU486 did not inhibit the increase in GR expression but rather enhanced the effect of glucocorticoids (Fig. 5A). Next, we examined the effect of RU486 on the increase in GR expression induced by long-term glucocorticoid treatment. Exposure to corticosterone (1 μM) and dexamethasone (1 μM) for 24 h reduced GR expression in the astrocytes. RU486 significantly prevented the glucocorticoid-induced reduction in GR expression (Fig. 5B). Taken together these

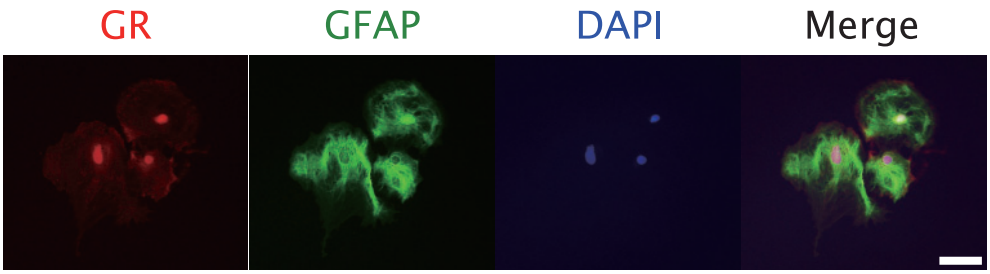

Fig. 1. Glucocorticoid receptor (GR) expression in astrocytes. GR and glial fibrillary acidic protein (GFAP) were detected by immunohistochemistry. Nuclei were stained with 4′,6-diamino-2-phenylindole (DAPI). Scale bar = 100 μm.

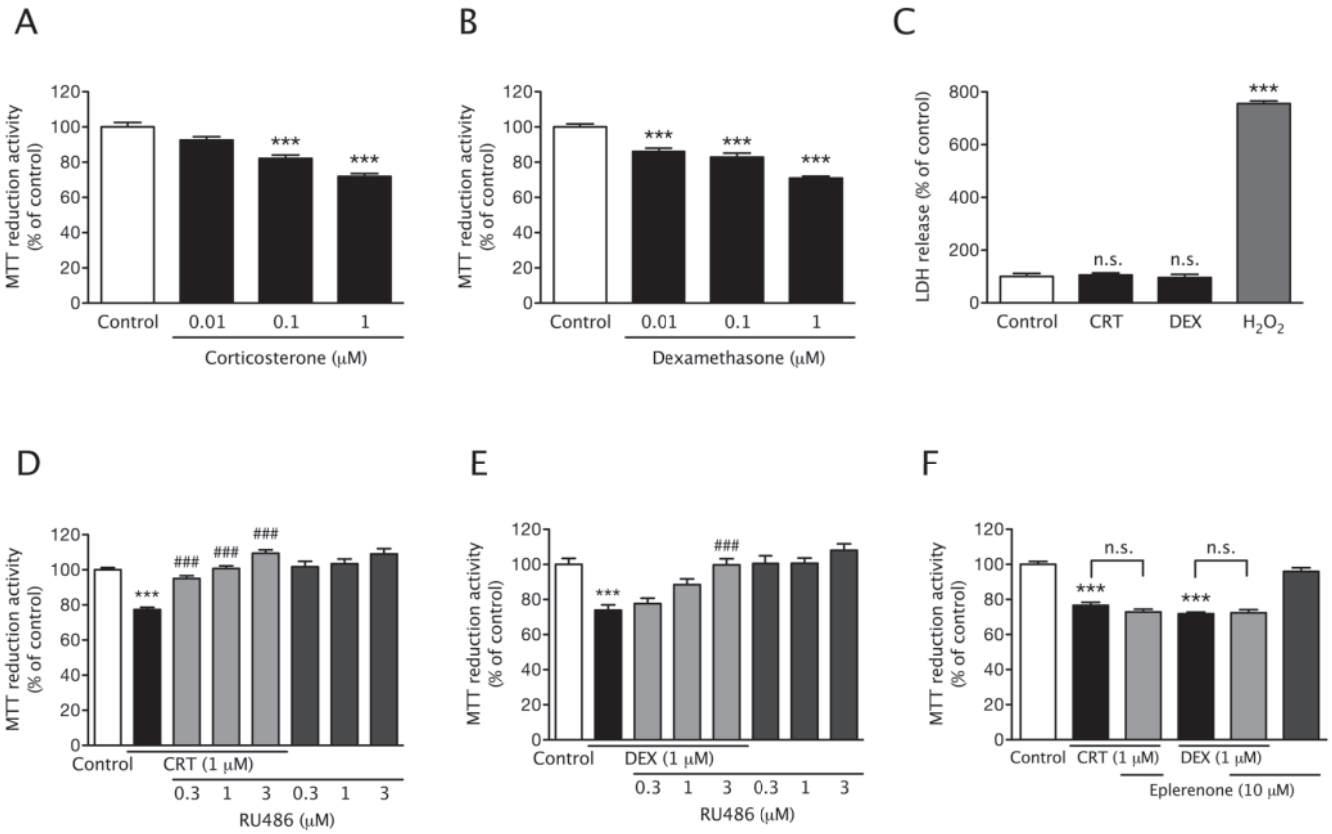

Fig. 2. Corticosterone (CRT) and dexamethasone (DEX) reduced the number of astrocytes via a glucocorticoid receptor but did not induce astrocytic damage. A, B: The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed 72 h after treatment with CRT (0.01 – 1 μM) and DEX (0.01 – 1 μM). C: The lactate dehydrogenase (LDH) assay was performed 72 h after treatment with CRT (1 μM) and DEX (1 μM) or 24 h after treatment with H_2O_2 (1 mM). D, E, F: The MTT assay was performed 72 h after treatment with CRT (1 μM); DEX (1 μM); RU486 (0.3 – 3 μM), a GR antagonist; and eplerenone (10 μM), a mineralcorticoid receptor antagonist. n = 4. ***$P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. CRT or DEX alone. n.s., not significant.

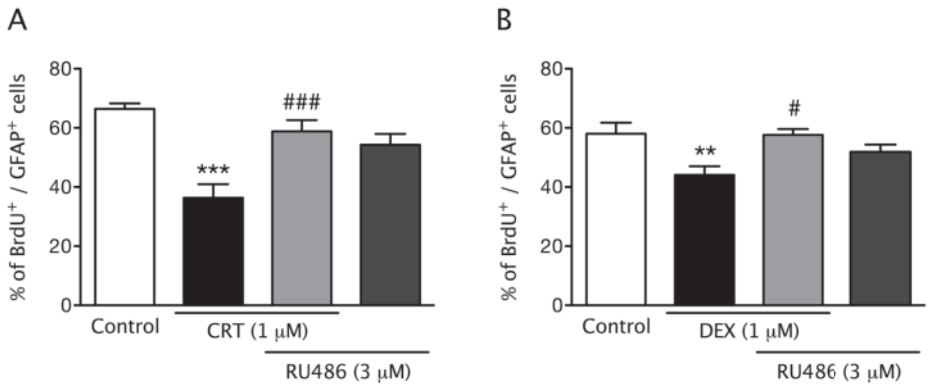

Fig. 3. Reduction in astrocyte proliferation by corticosterone (CRT) and dexamethasone (DEX) via a glucocorticoid receptor. Bromodeoxyuridine (BrdU)- and GFAP-positive cells were detected 48 h after treatment with CRT (1 μM), DEX (1 μM), and RU486 (3 μM) plus BrdU (1 μM). A: CRT, B: DEX. n = 8. **$P < 0.01$, ***$P < 0.001$ vs. control; $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ vs. CRT or DEX alone.

results suggest that glucocorticoids inhibited astrocyte proliferation and reduced GR expression mediated via GR.

GR knockdown led to a reduction in astrocyte numbers

To investigate the relationship between reduction in GR expression and inhibition of astrocyte proliferation by glucocorticoids, siRNA targeting GR was transfected

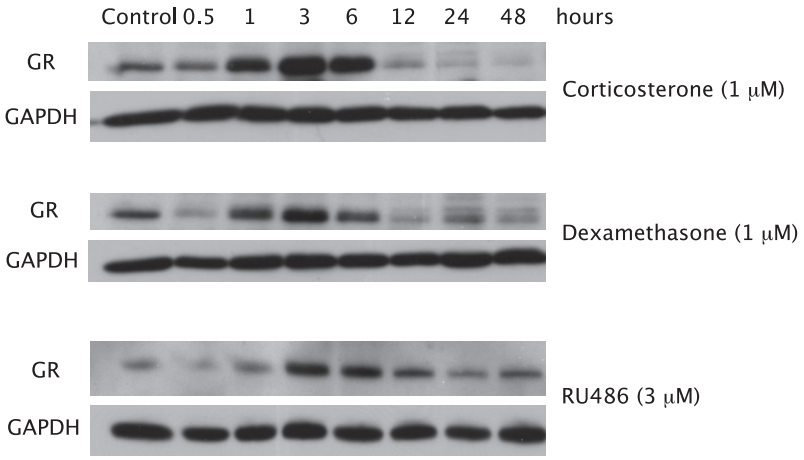

Fig. 4. Corticosterone, dexamethasone, and RU486 changed glucocorticoid receptor (GR) expression. GR and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were detected by western blotting at the indicated periods (0.5 – 48 h) after treatment with corticosterone (1 μM), dexamethasone (1 μM), or RU486 (3 μM).

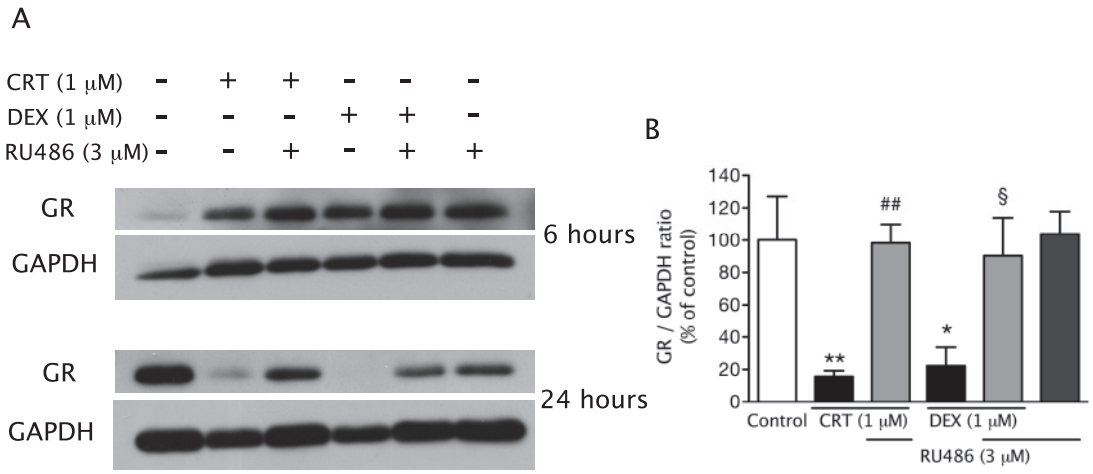

Fig. 5. RU486 affected changes in glucocorticoid receptor (GR) expression induced by corticosterone (CRT) and dexamethasone (DEX). A: GR and GAPDH were detected by western blotting after treatment with CRT (1 μM), DEX (1 μM), and RU486 (3 μM) for the indicated periods. B: Quantification of the GR/GAPDH ratio in astrocytes after 24-h treatment. n = 5 – 6. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{\#\#}P < 0.01$ vs. CRT alone; $^{\S}P < 0.05$ vs. DEX alone.

into the astrocytes. Immunoblotting showed a 40% reduction in expression of GR protein in the astrocytes transfected with GR siRNA (Fig. 6: A, B). The number of GR siRNA–transfected astrocytes was significantly reduced 120 h following transfection (Fig. 6C). Next, we examined the effect of GR knockdown on astrocyte proliferation. GR siRNA transfection significantly reduced the number of BrdU-positive astrocytes (Fig. 6D). These results suggest that reduction in GR expression in astrocytes resulted in the suppression of astrocyte proliferation.

Repeated ACTH administration caused a reduction in astrocyte numbers in vivo

We investigated the effects of high concentrations of glucocorticoids on astrocyte proliferation using a repeated ACTH administration rat model. First, we examined GR expression in the frontal cortex and hippocampus after 14 days of saline or ACTH administration (100 μg/day). GR expression in ACTH-administered rats was significantly decreased compared with that in saline-administered rats (Fig. 7: A, B). Next, we examined GFAP expression and measured the number of astrocytes and neurons in the frontal cortex following ACTH administration for 14 days. GFAP expression in the frontal cortex of ACTH-administered rats was reduced compared with that of saline-administered rats (Fig. 7C). Immunohistochemistry revealed that ACTH administration did not affect the number of neurons. In contrast, the number of astrocytes was significantly reduced. (Fig. 7: D, E).

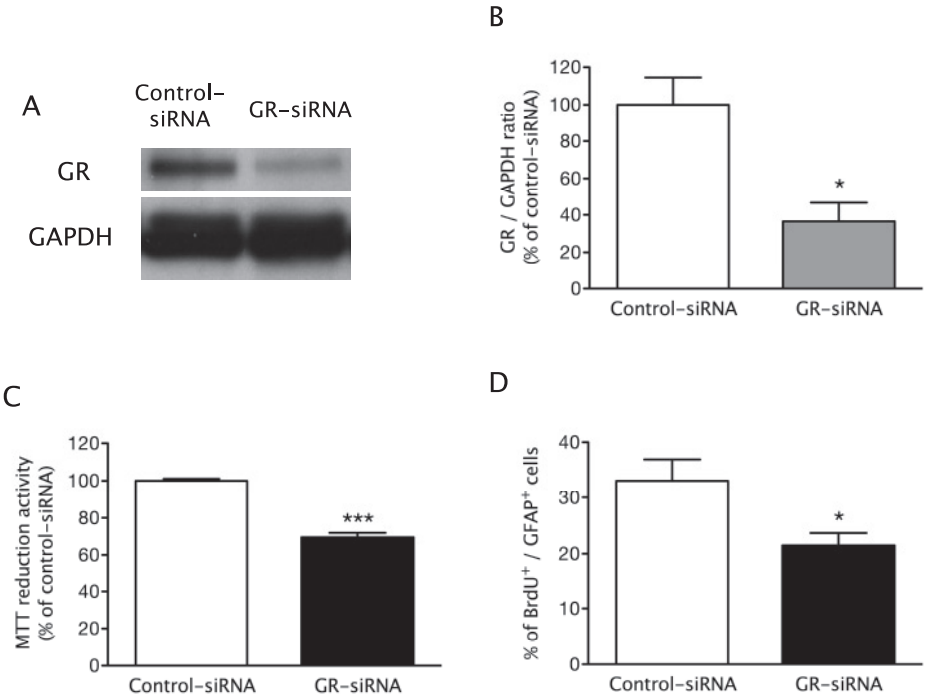

Fig. 6. Glucocorticoid receptor (GR) knockdown reduced astrocyte proliferation. A: Representative western blotting example of GR and GAPDH 96 h after siRNA transfection. B: Quantification of the GR/GAPDH ratio in panel A. n = 4. C: The MTT assay was performed 120 h after siRNA transfection. n = 4. D: BrdU-positive cells were detected 48 h after BrdU treatment (1 μM) 72 h after siRNA transfection. n = 8. $^{*}P < 0.05$, $^{***}P < 0.001$ vs. control siRNA.

These results suggest that secretion of high concentrations of glucocorticoids induced by repeated ACTH administration leads to a reduction in both GR expression and the number of astrocytes in vivo.

Discussion

This study investigated the mechanism by which glucocorticoids inhibit astrocyte proliferation. The results demonstrated a relationship between astrocyte proliferation and GR expression following treatment with glucocorticoids. This was demonstrated in an animal model (in vivo) as well as cultured astrocytes (in vitro). In summary, our results suggest that glucocorticoids reduce astrocyte proliferation by inducing a reduction in GR expression.

In the first experiment, the subcellular localization of GR was observed in cultured astrocytes, with GR found to be primarily expressed in the nuclei (Fig. 1). Neuronal GR performs functions other than gene transcription, for example, GR interacts with receptor tyrosine kinase for BDNF (trkB) and promotes glutamate release (20). In contrast, functions of astrocytic GR other than the function of binding to a glucocorticoid have not yet been reported. Based on our results, we assumed that astrocytic as well as neuronal GRs have some unknown functions in our culture conditions.

Corticosterone and dexamethasone reduced the number of astrocytes via GR by inhibiting astrocyte proliferation (Figs. 2 and 3). These results are consistent with those of a previous report that suggested that the neural cell adhesion molecule (NCAM) inhibited astrocyte proliferation via GR (16). In neurons and astrocytes, GR expression has also been shown to change with glucocorticoid administration in vitro (19). The mechanisms by which GR expression affects astrocyte function are largely unknown. We showed that short-term (1 – 6 h) glucocorticoid treatment increased GR expression, whereas long-term (> 12 h) treatment decreased GR expression. RU486 also increased GR expression in a short term; however, this expression did not decrease subsequently (Fig. 4). We examined the effect of RU486 on the glucocorticoid-induced change in GR expression. GR expression increased by glucocorticoid treatment was enhanced by RU486, whereas GR expression reduced by glucocorticoid treatment was inhibited by RU486 (Fig. 5).

We hypothesized that reduced GR expression following long-term glucocorticoid treatment plays a crucial role in astrocyte proliferation. To validate this hypothesis, we used siRNA to silence GR. GR knockdown reduced the number of astrocytes by inhibiting their proliferation (Fig. 6). GR antagonism by RU486 did not induce promotion of astrocyte proliferation. We suppose that this is because RU486 does not decrease GR expression in astrocyte. Thus, we propose that prolonged reduction in GR expression inhibits proliferation of cultured astrocytes. However, another study using murine macrophage

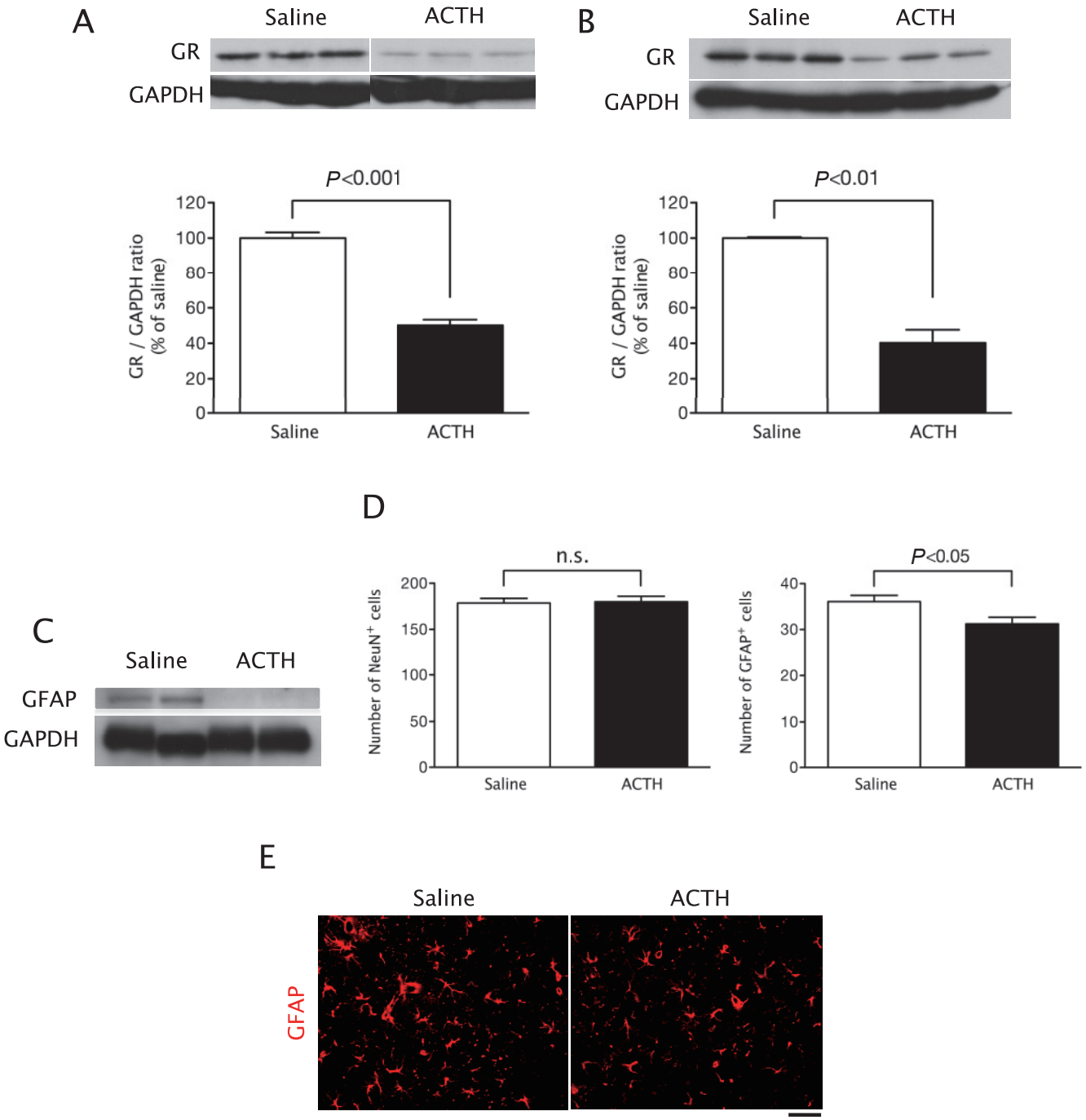

Fig. 7. Repeated adrenocorticotropic hormone (ACTH) administration decreased glucocorticoid receptor (GR) expression and the number of astrocytes in vivo. A, B: GR expression in the frontal cortex (A) and hippocampus (B) after 14 days of saline or ACTH administration. The bottom graphs show the quantified data of the GR/GAPDH ratio. n = 3. C: GFAP and GAPDH were detected by western blotting in the frontal cortex after 14 days of repeated ACTH administration. D: Neuronal nuclei (NeuN)- or GFAP-positive cells were stained by immunohistochemistry and their numbers calculated from 30 slices. E: Representative pictures show GFAP-positive cells in the prefrontal cortex of rats administered with saline or ACTH for 14 days. Scale bar = 50 μm.

cells reported that GR knockdown promoted cell proliferation, which is in contrast to our results (21). This discrepancy suggests that the relationship between GR expression and cell proliferation may depend on cell type. The mechanisms by which GR regulates cell proliferation should be determined in future research.

We also investigated the effect of glucocorticoids on the number of astrocytes and GR expression in vivo using a repeated ACTH administration model characterized by excessive glucocorticoid release and hyperactivation of the HPA axis. Fourteen days of ACTH administration reduced GR expression in both the frontal cortex and hippocampus. Furthermore, ACTH also reduced GFAP expression and the number of astrocytes in the frontal cortex (Fig. 7). Previous study using hippocampal astrocyte suggested dexamethasone decreased GFAP expression (22). Therefore, there are only modest GFAP cell number changes even though reduction of GFAP expression. This in vivo study demonstrated that glucocorticoid treatment also decreased the number of astrocytes in vivo and suggests that reduction in GR expression inhibits cell proliferation in the prefrontal cortex, similar to that observed in the in vitro study. In vivo study, glucocorticoid exposure by ACTH may inhibit proliferation of astrocytes and its progenitor cells. It has been previously suggested that chronic ACTH administration in rats might provide a model of antidepressant-treatment–resistant depression (23).

Mice that are heterozygous for the GR-knockout are believed to be a model of depression and exhibit reduced hippocampal neurogenesis (24), while mice with reduced GR expression in the frontal cortex show dysfunction of the HPA axis and depression-like behaviors (13). These in vivo reports revealed that compromised GR function could elicit depression-like behaviors. Therefore, we propose that dysfunction of GR, including the reduction in GR expression, inhibits astrocyte proliferation resulting in depression-like symptoms. Consistent with this proposal, a previous study of a rat model of depression identified a reduction in the number of astrocytes in the prefrontal cortex, hippocampus, and amygdala (9). Furthermore, previous reports suggested that chronic stress could decrease the number of astrocytes in the frontal cortex and hippocampus (10, 25). Currently, there is no direct evidence that a reduction in the number of astrocytes causes the pathogenesis of depression and thus further studies are required.

Serotonin reuptake inhibitors are commonly used in the clinical treatment of depression. Fluoxetin, a selective serotonin reuptake inhibitor, has been shown to restore the number of astrocytes in the hippocampus following their reduction by chronic stress (25). Regarding antidepressant drugs and GR, previous reports suggested that antidepressants increase GR expression in various brain regions and enhance neurogenesis by activating GR (26 – 28). Another report suggested that gliotoxin injection into the hippocampus inhibited the effect of an antidepressant in a rat model of depression (29). Therefore, altered astrocyte proliferation appears to play an important role in the pathogenesis of depression and could be a potential target for therapy.

Taken together, our results and previous reports suggest that chronic stress causes dysfunction of the HPA axis and subsequent excessive glucocorticoid release and that glucocorticoid exposure decreases GR expression, ultimately leading to a reduction in the number of astrocytes.

References

1 Markham JA, Morris JR, Juraska JM. Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. Neuroscience. 2007;144:961–968.

2 Li J, Ding YH, Rafols JA, Lai Q, McAllister JP 2nd, Ding Y. Increased astrocyte proliferation in rats after running exercise. Neurosci Lett. 2005;386:160–164.

3 Mandyam CD, Wee S, Eisch AJ, Richardson HN, Koob GF. Methamphetamine self-administration and voluntary exercise have opposing effects on medial prefrontal cortex gliogenesis. J Neurosci. 2007;27:11442–11450.

4 Rapanelli M, Frick LR, Zanutto BS. Learning an operant conditioning task differentially induces gliogenesis in the medial prefrontal cortex and neurogenesis in the hippocampus. PLoS One. 2011;6:e14713.

5 Nixdorf-Bergweiler BE, Albrecht D, Heinemann U. Developmental changes in the number, size, and orientation of GFAP-positive cells in the CA1 region of rat hippocampus. Glia. 1994;12:180–195.

6 Rietze R, Poulin P, Weiss S. Mitotically active cells that generate neurons and astrocytes are present in multiple regions of the adult mouse hippocampus. J Comp Neurol. 2000;424:397–408.

7 Mao L, Wang JQ. Gliogenesis in the striatum of the adult rat: alteration in neural progenitor population after psychostimulant exposure. Brain Res Dev Brain Res. 2001;130:41–51.

8 Rajkowska G, Miguel-Hidalgo JJ. Gliogenesis and glial pathology in depression. CNS Neurol Disord Drug Targets. 2007;6: 219–233.

9 Gosselin RD, Gibney S, O'Malley D, Dinan TG, Cryan JF. Region specific decrease in glial fibrillary acidic protein immunoreactivity in the brain of a rat model of depression. Neuroscience. 2009;159:915–925.

10 Banasr M, Duman RS. Glial loss in the prefrontal cortex is sufficient to induce depressive-like behaviors. Biol Psychiatry. 2008;64:863–870.

11 Keller J, Flores B, Gomez RG, Solvason HB, Kenna H, Williams GH, et al. Cortisol circadian rhythm alterations in psychotic major depression. Biol Psychiatry. 2006;60:275–281.

12 Aihara M, Ida I, Yuuki N, Oshima A, Kumano H, Takahashi K, et al. HPA axis dysfunction in unmedicated major depressive disorder and its normalization by pharmacotherapy correlates with alteration of neural activity in prefrontal cortex and limbic/paralimbic regions. Psychiatry Res. 2007;155:245–256.

13 Boyle MP, Brewer JA, Funatsu M, Wozniak DF, Tsien JZ, Izumi Y, et al. Acquired deficit of forebrain glucocorticoid receptor produces depression-like changes in adrenal axis regulation and behavior. Proc Natl Acad Sci U S A. 2005;102:473–478.

14 Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, et al. Mice with genetically altered glucocorticoid receptor

expression show altered sensitivity for stress-induced depressive reactions. J Neurosci. 2005;25:6243–6250.
15 Schmidt MV, Sterlemann V, Wagner K, Niederleitner B, Ganea K, Liebl C, et al. Postnatal glucocorticoid excess due to pituitary glucocorticoid receptor deficiency: differential short- and long-term consequences. Endocrinology. 2009;150:2709–2716.
16 Crossin KL, Tai MH, Krushel LA, Mauro VP, Edelman GM. Glucocorticoid receptor pathways are involved in the inhibition of astrocyte proliferation. Proc Natl Acad Sci U S A. 1997;94:2687–2692.
17 Yu S, Yang S, Holsboer F, Sousa N, Almeida OF. Glucocorticoid regulation of astrocytic fate and function. PLoS One. 2011;6:e22419.
18 Kume T, Ito R, Taguchi R, Izumi Y, Katsuki H, Niidome T, et al. Serofendic acid promotes stellation induced by cAMP and cGMP analogs in cultured cortical astrocytes. J Pharmacol Sci. 2009;109:110–118.
19 Bohn MC, O'Banion MK, Young DA, Giuliano R, Hussain S, Dean DO, et al. In vitro studies of glucocorticoid effects on neurons and astrocytes. Ann N Y Acad Sci. 1994;746:243–259.
20 Numakawa T, Kumamaru E, Adachi N, Yagasaki Y, Izumi A, Kunugi H. Glucocorticoid receptor interaction with TrkB promotes BDNF-triggered PLC-gamma signaling for glutamate release via a glutamate transporter. Proc Natl Acad Sci U S A. 2009;106:647–652.
21 Zhu XY, Liu YJ, Lu J, Xu RB. Knockdown of glucocorticoid receptor expression by RNA interference promotes cell proliferation in murine macrophage RAW264.7 cells. J Steroid Biochem Mol Biol. 2004;92:375–382.
22 Niu H, Hinkle DA, Wise PM. Dexamethasone regulates basic fibroblast growth factor, nerve growth factor and S100beta expression in cultured hippocampal astrocytes. Brain Res Mol Brain Res. 1997;51:97–105.
23 Kitamura Y, Araki H, Gomita Y. Influence of ACTH on the effects of imipramine, desipramine and lithium on duration of immobility of rats in the forced swim test. Pharmacol Biochem Behav. 2002;71:63–69.
24 Kronenberg G, Kirste I, Inta D, Chourbaji S, Heuser I, Endres M, et al. Reduced hippocampal neurogenesis in the GR(+/−) genetic mouse model of depression. Eur Arch Psychiatry Clin Neurosci. 2009;259:499–504.
25 Czéh B, Simon M, Schmelting B, Hiemke C, Fuchs E. Astroglial plasticity in the hippocampus is affected by chronic psychosocial stress and concomitant fluoxetine treatment. Neuropsychopharmacology. 2006;31:1616–1626.
26 Peiffer A, Veilleux S, Barden N. Antidepressant and other centrally acting drugs regulate glucocorticoid receptor messenger RNA levels in rat brain. Psychoneuroendocrinology. 1991;16:505–515.
27 Okugawa G, Omori K, Suzukawa J, Fujiseki Y, Kinoshita T, Inagaki C. Long-term treatment with antidepressants increases glucocorticoid receptor binding and gene expression in cultured rat hippocampal neurones. J Neuroendocrinol. 1999;11:887–895.
28 Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, et al. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry. 2011;16:738–750.
29 Iwata M, Shirayama Y, Ishida H, Hazama GI, Nakagome K. Hippocampal astrocytes are necessary for antidepressant treatment of learned helplessness rats. Hippocampus. 2011;21:877–884.