Biochemical and Biophysical Research Communications 372 (2008) 243–248

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Dexamethasone coordinately regulates angiopoietin-1 and VEGF: A mechanism of glucocorticoid-induced stabilization of blood–brain barrier

Hyongbum Kim [a,b,1], Jung Min Lee [a,c,1], Jae Sun Park [a,d], Sangmee Ahn Jo [a], Yong-Ou Kim [a], Chan-Wha Kim [c], Inho Jo [e,f,*]

[a] Department of Biomedical Sciences, National Institute of Health, Seoul, South Korea
[b] Department of Medical Engineering, Yonsei University College of Medicine, Seoul, South Korea
[c] Department of Biotechnology, Korea University School of Life Sciences and Biotechnology, Seoul, South Korea
[d] Department of Biology, Kyung Hee University College of Science, Seoul, South Korea
[e] Department of Molecular Medicine, Ewha Womans University School of Medicine, Mok-6-dong, Yangchun-gu, Seoul 158-710, South Korea
[f] Ewha Medical Research Institute, Ewha Womans University School of Medicine, Seoul, South Korea

ARTICLE INFO

Article history:
Received 6 May 2008
Available online 15 May 2008

Keywords:
Glucocorticoids
Dexamethasone
Endothelial cells
Astrocytes
Pericytes
Blood–brain barrier
Vascular endothelial growth factor
Angiopoietin-1
Angiopoietin-2

ABSTRACT

Glucocorticoids stabilize the blood–brain barrier (BBB), leading to attenuation of vasogenic brain edema. However, the action mechanism of glucocorticoids has been poorly elucidated. To elucidate the mechanism, we investigated whether dexamethasone (Dex), a synthetic glucocorticoid hormone, regulates the levels of key permeability regulating factors such as angiopoietin-1, angiopoietin-2, and vascular endothelial growth factor (VEGF) in the three types of cells comprising BBB. Dex increased the level of angiopoietin-1 mRNA and protein and decreased VEGF mRNA and protein in brain astrocytes and pericytes, but not in endothelial cells. The mRNA and protein of angiopoietin-2 were detected only in endothelial cells and not regulated by Dex. The Dex-induced regulation of angiopoietin-1 and VEGF was inhibited by RU486, suggestive of glucocorticoid receptor mediation. The mRNA stability of angiopoietin-1 and VEGF was not changed by Dex treatment, implying that Dex increases angiopoietin-1 and decreases VEGF through transcriptional regulation. This is the first study showing the coordinate regulation of angiopoietin-1 and VEGF by glucocorticoids, suggesting a novel mechanism underlying glucocorticoids-induced stabilization of BBB.

The blood–brain barrier (BBB) is a diffusion barrier essential for the normal function of the central nervous system (CNS) [1,2]. The BBB restricts the nonspecific flux of ions, proteins, and other harmful substances and selectively allows the uptake of essential molecules from the blood into the CNS microenvironment. It is mainly comprised of three cellular elements of the brain microvasculature: endothelial cells, astrocyte end-feet, and pericytes [1–3]. Endothelial cells are directly responsible for formation of barriers, while astrocytes and pericytes regulate the barrier function of endothelial cells. Compared to endothelial cells in other tissues, the BBB endothelial cells have more extensive tight junctions, sparse pinocytic vesicular transport, and no fenestrations [1,3]. Astrocytic end-feet tightly ensheathe the vessel wall and appear to be crucial for the induction and maintenance of the tight junction barrier, although astrocytes themselves are not thought to have a barrier function [1]. Pericytes embrace capillary endothelium, contributing to maturation of vessels [4] and are regarded as one of the critical components for establishment of an *in vitro* BBB model [5].

The key factors that regulate the stability of BBB are vascular endothelial growth factor (VEGF), angiopoietin-1 (Ang-1), and angiopoietin-2 (Ang-2) [6–9]. VEGF is a key regulator of angiogenesis and was initially identified as vascular permeability factor owing to its potent property of increasing the permeability of vascular wall [10]. VEGF disrupts endothelial cell–cell junctions, leading to increased vascular permeability, which results in breakdown of BBB [6,11]. Angiopoietins are ligands for Tie-2, which is a receptor tyrosine kinase expressed on endothelial cells and hematopoietic stem cells [12–14]. Among angiopoietins, the best characterized are Ang-1 and Ang-2 [14]. Ang-1 induces autophosphorylation of Tie-2, while Ang-2 acts as a natural antagonist in endothelial cells in the presence of Ang-1 [15]. Ang-1 precludes blood vessels from leaking [14,16], while Ang-2 destabilizes the vessels. Ang-1 secreted by brain astrocytes and pericytes contributes to formation

* Corresponding author. Address: Department of Molecular Medicine, Ewha Womans University School of Medicine, Mok-6-dong, Yangchun-gu, Seoul 158-710, South Korea. Fax: +82 2 2650 5786.
E-mail address: inhojo@ewha.ac.kr (I. Jo).
[1] The first two authors contributed equally to this paper.

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.05.025

of the BBB [7,8], whereas Ang-2 directly results in breakdown of the BBB [9].

Pathological conditions such as ischemia, brain tumors, or head injury are accompanied by breakdown of the BBB and consequent increase in permeability [6,17]. As a result, plasma components leak from the blood into the extracellular space of the brain, which causes the development of vasogenic cerebral edema. Glucocorticoids are the mainstay of the treatment for vasogenic brain edema, and dexamethasone (Dex) is the most commonly used glucocorticoid [6]. However, the action mechanism of Dex on the stability of BBB has yet to be elucidated.

In an attempt to elucidate the action mechanism of glucocorticoids for stabilization of the BBB, we investigated if Dex, a synthetic glucocorticoid, regulates the expression levels of three key factors regulating BBB, i.e., VEGF, Ang-1, and Ang-2 in BBB-comprising cells such as human brain astrocytes (HBAs), human brain pericytes (HBPs), and human brain microvascular endothelial cells (HBMECs). Here, we demonstrate for the first time that Dex upregulates angiopoietin-1 and downregulates VEGF in HBAs and HBPs, suggesting a novel mechanism underlying Dex-induced stabilization of BBB.

Materials and methods

Cell culture. Primarily cultured HBAs, HBPs, and HBMECs were purchased from Cell Systems (Kirkland, WA) and cultured in CSC media (Cell Systems) in the presence of attachment factors (Cell Systems). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

Real-time reverse transcription-polymerase chain reaction (RT-PCR). Cells were stimulated with various concentrations of Dex (Sigma Chemical, St. Louis, MO) for 24 h or indicated time. Total RNA was isolated from the cells using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instruction. The RT reaction was carried out as previously described [18]. Quantitative real-time PCR was performed using the 7900 HT sequence detection system (Applied Biosystems, Foster City, CA) and SYBR Green master mix (Applied Biosystems). Primer pairs for the real-time PCR were as follows: for human β-actin, forward 5′-GCCCAGTC CTCTCCCAAGTC-3′, reverse 5′-GGCACGAAGGCTCATCATTC-3′; for human VEGF, forward 5′-GTTTCGGGAACCAGATCTCTCA-3′, reverse 5′-GGACTGTTCTGTCGATGGTGATG-3′; for human Ang-1, forward 5′-GGAAGGGAACCGAGCCTATT-3′, reverse 5′-TTCCTGCTGTCCCAGT GTGA-3′; for human Ang-2, forward 5′-CAGCATCAGCCAACCAGGA A-3′, reverse 5′-CAAACCACCAGCCTCCTGTTAG-3′.

Determination of protein levels of Ang-1, Ang-2, and VEGF in the cell culture supernatants. After exposure to Dex, the supernatants of cell culture were collected, centrifuged at 3000*g* for 5 min to remove any cellular debris and stored at −70 °C until assayed. Ang-1, Ang-2, and VEGF concentrations were determined using ELISA kits (R&D systems, Inc., Minneapolis, MN) according to the manufacturer's instruction.

RU486 inhibition test. Cells were stimulated with various concentrations of Dex in the presence of various concentrations of

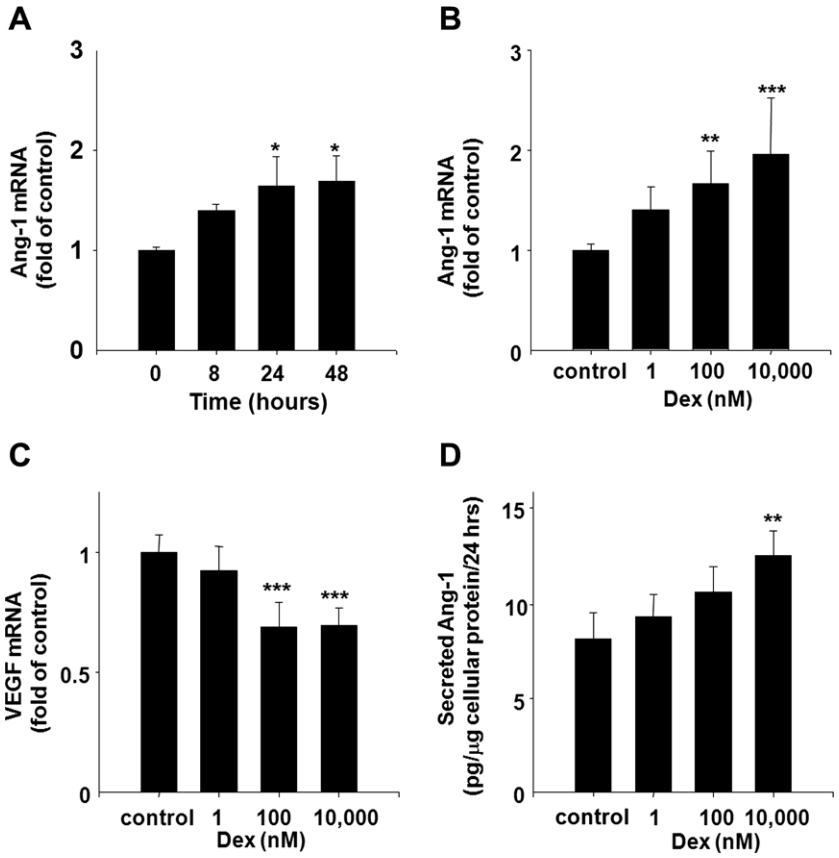

Fig. 1. Effect of Dex on the expression levels of Ang-1 and VEGF in astrocytes. Human brain astrocytes were stimulated with Dex at 100 nM for indicated times (A) or at various concentrations for 24 h (B–D). (A–C) Total RNA was isolated using Trizol. The mRNA levels of VEGF, Ang-1, and Ang-2 were determined using real-time RT-PCR and normalized to those of β-actin. Each bar represents the mean Ang-1 (A and B) or VEGF (C) mRNA level ±SD relative to solvent control-treated cells. Dex significantly decreased VEGF mRNA and increased Ang-1 mRNA ($^{*}P < 0.05$, $^{*}P < 0.01$, $^{***}P < 0.001$ vs solvent control, $n = 3$–7 (A), $n = 9$ (B), $n = 7$ (C)). (D) The concentrations of Ang-1 in the supernatants were determined using ELISA. Each bar represents the mean amount of secreted Ang-1 ±SD normalized to that of total cellular protein. Dex significantly increased Ang-1 protein in a dose-dependent manner ($^{**}P < 0.01$ vs solvent control, $n = 4$).

RU486 for 24 h and the supernatants were collected and analyzed as described above.

mRNA stability analysis. Cells were exposed to 100 μM Dex for 24 h and actinomycin D (10 μg/ml) was added to stop RNA synthesis. Total RNA was isolated at the indicated time points and analyzed by real-time RT-PCR as described above.

Statistical analysis. The results are expressed as means ± SD. Significance was determined by ANOVA followed by multiple comparisons by Tukey's method using SPSS for Windows (SPSS Inc., Chicago, IL). We took $P < 0.05$ to be statistically significant.

Results

The expression levels of Ang-1, Ang-2, and VEGF in HBPs, HBAs, and HBMECs

To determine the basal expression levels of Ang-1, Ang-2, and VEGF in the three kinds of cells responsible for the formation of the BBB, we quantified secreted proteins in the cell culture supernatants. In HBAs and HBMECs, only Ang-1 and Ang-2 proteins were significantly detected, respectively (Supplementary

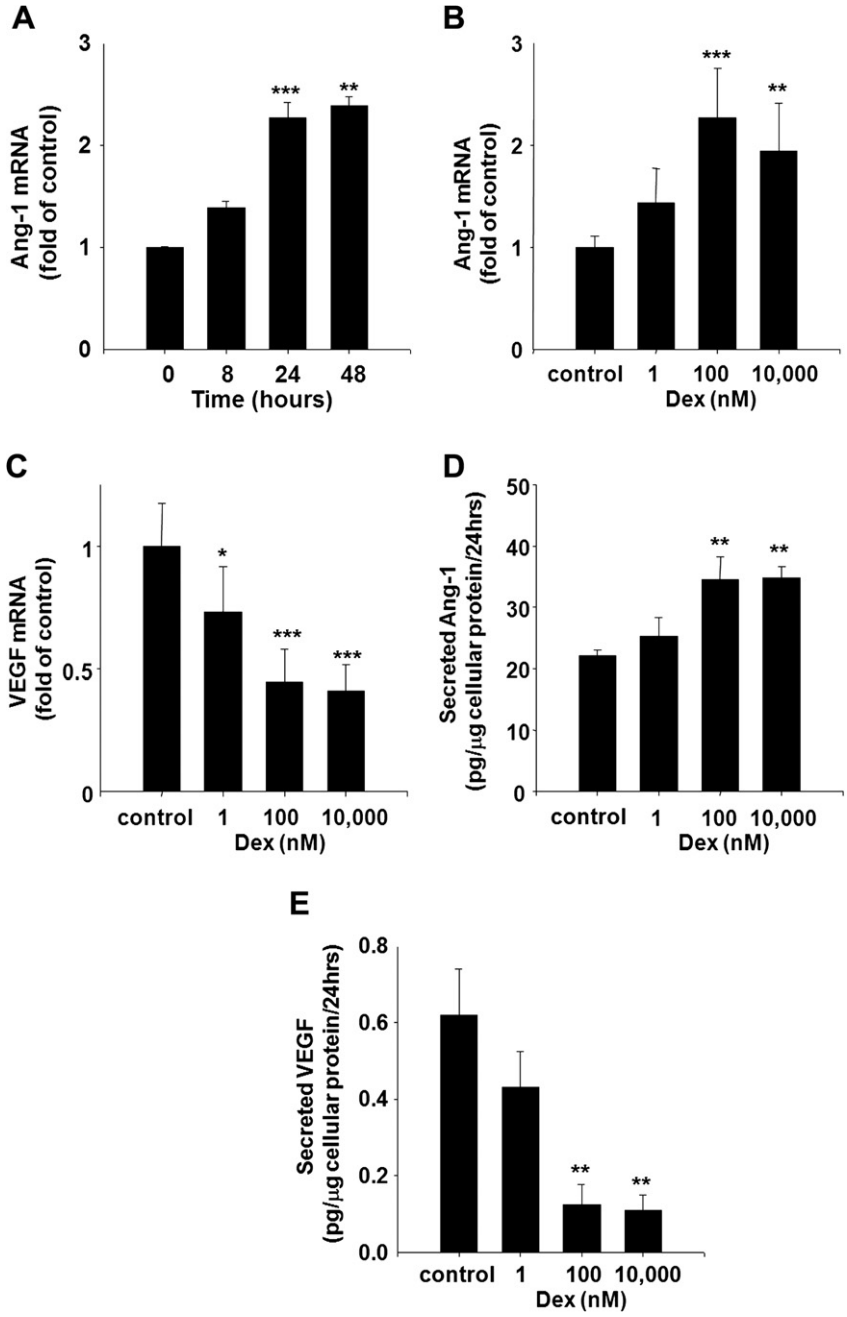

Fig. 2. Effect of Dex on the expression levels of Ang-1 and VEGF in pericytes. Human brain pericytes were stimulated with Dex at 100 nM for indicated times (A) or at various concentrations for 24 h (B–E). (A–C) Ang-1 and VEGF mRNA were determined as described in the legend to Fig. 1. Each bar represents the mean Ang-1 (A,B) or VEGF (C) mRNA level ±SD relative to solvent control-treated cells. Dex significantly increased Ang-1 and decreased VEGF mRNA ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs solvent control, $n = 3$–10 (A), $n = 7$–10 (B), $n = 7$ (C)). (D,E) The concentrations of Ang-1 and VEGF in the supernatants were determined using ELISA. Each bar represents the mean amount of secreted Ang-1 (D) and VEGF (E) ±SD normalized to that of total cellular protein. Dex significantly increased Ang-1 protein and decreased VEGF protein in a dose-dependent manner ($^{**}P < 0.01$ vs solvent control, each $n = 3$).

Fig. 1). In HBPs, we detected significant amount of Ang-1 and VEGF protein, but not Ang-2. In case of mRNA levels, real-time RT-PCR revealed that Ang-1 and VEGF mRNA were expressed in all of the three types of cells although the expression level of Ang-1 in HBMECs was ~50- and ~70-fold lower than those in HBAs and HBPs, respectively (data not shown). In contrast, Ang-2 mRNA was detectable in HBMECs, but not in HBAs and HBPs. Further studies were carried out based on these expression levels.

Dex increases Ang-1 mRNA and protein but decreases VEGF mRNA in HBAs

To investigate whether Dex affects the level of Ang-1 and VEGF in HBAs, we treated HBAs with 100 nM of Dex for various times and determined the level of Ang-1 mRNA by real-time RT-PCR. Dex upregulated Ang-1 mRNA in HBAs in a time-dependent manner (Fig. 1A) and the maximal response of 1.64 ± 0.30 fold was obtained at 24 h. Then, we stimulated HBAs with various concentrations of Dex for 24 h and found that Dex increase Ang-1 also in a dose-dependent manner with maximal response of 1.70 ± 0.33 fold at 100 nM and 2.0 ± 0.6 at 10 μM (Fig. 1B). In contrast, Dex decreased VEGF mRNA in HBAs in a dose-dependent manner to 0.69 ± 0.11 fold at 100 nM and 0.70 ± 0.07 at 10 μM (Fig. 1C). Because Ang-1 protein is expressed in HBAs, we also examined the protein levels of Ang-1 after treatment with Dex. As in mRNA level, Dex increased Ang-1 protein in a dose-dependent manner (Fig. 1D). Neither mRNA nor protein of Ang-2 was detected in HBAs.

Dex increases Ang-1 mRNA and protein but decreases VEGF mRNA and protein in HBPs

Next, we examined if Dex affects the mRNA and protein levels of Ang-1 and VEGF in HBPs. As in HBAs, Dex also increased Ang-1 mRNA level in a time- and dose-dependent manner with maximal response of 2.3 ± 0.5 fold at 100 nM Dex for 24 h and decreased VEGF mRNA level to 0.45 ± 0.13 fold at 100 nM and 0.41 ± 0.11 at 10 μM in HBPs (Fig. 2A–C). As in mRNA level, Dex also decreased Ang-1 and VEGF protein in a dose-dependent manner (Fig. 2D and E). Neither mRNA nor protein of Ang-2 was detected in HBPs.

Dex does not change the levels of Ang-1, Ang-2, and VEGF in HBMECs

Various concentrations of Dex failed to significantly affect the mRNA levels of VEGF, Ang-1, and Ang-2 in HBMECs (Fig. 3). Dex did not influence the Ang-2 protein levels in HBMECs, either.

RU486 completely inhibits the Dex-induced regulation of Ang-1 and VEGF

Most effects of glucocorticoids on cells are mediated by activation of glucocorticoid receptor (GR) [19]. To examine whether Dex-induced increase in Ang-1 and decrease in VEGF are GR-mediated, we stimulated HBPs with 100 nM Dex in the presence of RU486, a GR antagonist. Addition of equimolar concentration of RU486 significantly attenuated the effects of Dex ($P < 0.001$ for Ang-1, $P < 0.05$ for VEGF) and 100-fold higher concentration of RU486 led to complete abrogation (Fig. 4). Various concentrations of

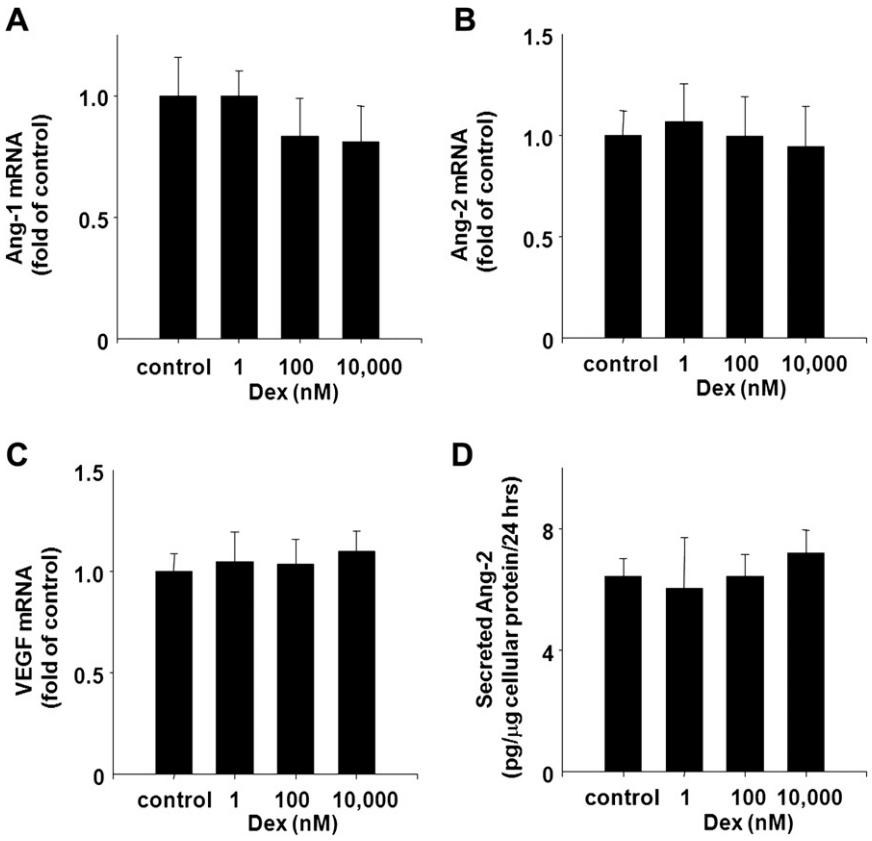

Fig. 3. Effect of Dex on the expression levels of Ang-1, Ang-2, and VEGF in endothelial cells. Human brain microvascular endothelial cells were stimulated with Dex at various concentrations for 24 h. (A-C) Ang-1, Ang-2, and VEGF mRNA were determined as described in the legend to Fig. 1. Each bar represents the mean Ang-1 (A), Ang-2 (B), and VEGF (C) mRNA level ±SD relative to solvent control-treated cells. The mRNA levels of Ang-1, Ang-2, and VEGF were not affected by Dex (each $n = 7–9$). (D) The concentrations of Ang-2 in the supernatants were determined using ELISA. Each bar represents the mean amount of secreted Ang-2 ± SD normalized to that of total cellular protein. Various concentrations of Dex did not change the level of Ang-2 protein ($n = 3$).

RU486 in the absence of Dex did not affect Ang-1 and VEGF protein levels in HBPs. Dose-dependent inhibition by RU486 indicates that Dex upregulates Ang-1 and downregulates VEGF through a GR-mediated mechanism.

Dex does not affect the mRNA stability of Ang-1 and VEGF

To elucidate the mechanism by which Dex upregulates Ang-1 mRNA and downregulates VEGF mRNA, we measured the mRNA stability of Ang-1 and VEGF in HBPs. Ang-1 mRNA was very stable and remained unchanged for 12 h (Fig. 4C), which is compatible with the previous report of high stability of Ang-1 mRNA in retinal pigment epithelial cells [20]. In contrast, VEGF mRNA decreased rapidly under the same experimental conditions (Fig. 4D). Note that Dex treatment did not affect Ang-1 and VEGF mRNA stability in HBPs, implying that the regulations of Ang-1 and VEGF by Dex are mediated by transcriptional activation and inhibition, respectively.

Discussion

In our study, we showed that Dex coordinately regulates the expression of Ang-1, Ang-2, and VEGF in three types of cells comprising BBB. The most salient findings in the current study are as follows. First, we demonstrated, for the first time, that Dex increased Ang-1 and decreased VEGF levels in HBAs and HBPs, but not in HBMECs, suggesting a novel mechanism of Dex-induced stabilization of BBB. Second, Dex-induced regulation of Ang-1 and VEGF was attenuated by RU486, indicative of a GR mediation. Third, Dex did not change the mRNA stability of Ang-1 and VEGF, implying transcriptional regulation of Ang-1 and VEGF by Dex.

Most of the effects of glucocorticoids on cells are through GR, which is activated by binding to its specific ligands such as glucocorticoid hormone and Dex [19]. In our study, RU486, a GR antagonist, completely inhibited the Dex-induced regulation of Ang-1 and VEGF, indicating the mediation by GR (Fig. 4A and B). The well known mode of action of activated GR is through the regulation of gene transcription [19,21] although the effects of GR without transcriptional modulation are recently being revealed [22]. In this study, the transcriptional regulation of Ang-1 and VEGF may mediate the upregulation of Ang-1 and downregulation of VEGF by Dex because Dex did not affect mRNA stability of Ang-1 and VEGF (Fig. 4C and D).

Ang-2 mRNA was not detected in either HBAs or HBPs, while the level of Ang-1 mRNA in HBMECs was observed at much lower levels than in HBAs and HBPs. This is in fair agreement with previous reports stating that Ang-1 is expressed mainly in mesenchymal cells, such as pericytes, astrocytes, smooth muscle cells, and solid tumor cells of mesenchymal origin [7,23–25], whereas Ang-2 is induced in endothelial cells, particularly within actively sprouting vessels or zones of vessel regression [15,25]. In addition, the expression of Ang-1 in endothelial cells seems to vary depending on the organs and types of endothelial cells: Ang-1 mRNA is barely expressed in bovine aortic endothelial cells [26], whereas rat pituitary endothelial cells and bovine choroidal microvascular endothelial cells express Ang-1 [27,28]. The difference in Ang-1

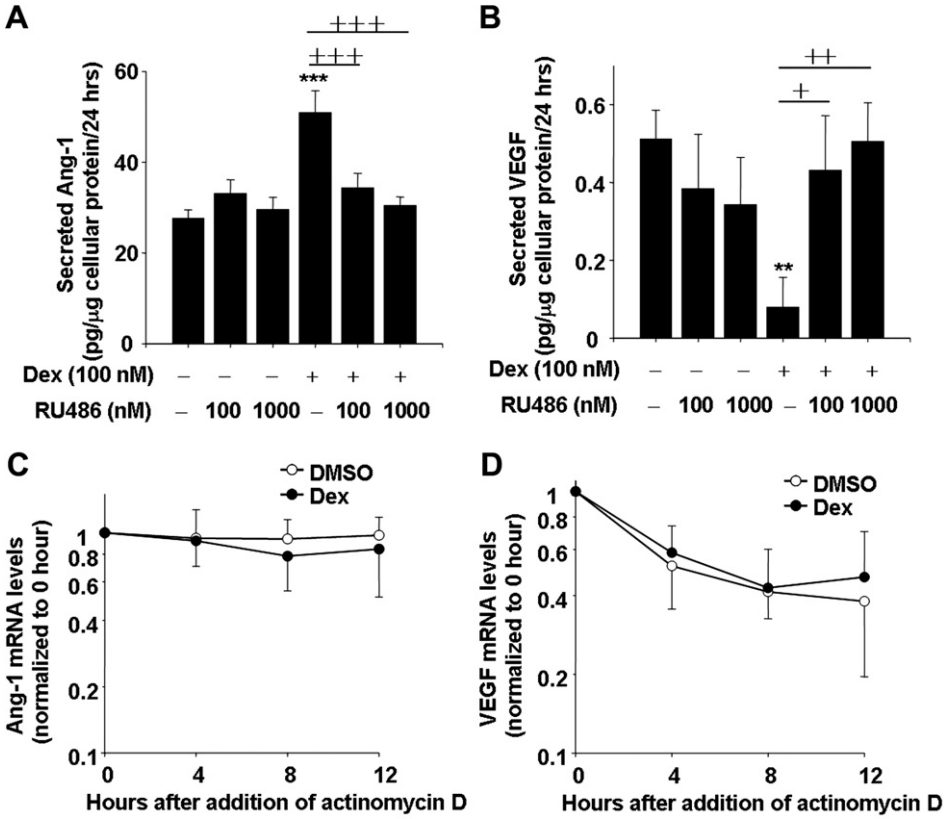

Fig. 4. The mechanism underlying the Dex-induced regulation of Ang-1 and VEGF. (A,B) HBPs were treated with 100 nM Dex in the presence or absence of RU486, a GR antagonist for 24 h. The concentrations of Ang-1 in the supernatants were determined using ELISA. Each bar represents the mean amount of secreted Ang-1 (A) and VEGF (B) ±SD normalized to that of total cellular protein. The Dex-induced increase in Ang-1 and decrease in VEGF ($^{***}P < 0.001$, $^{**}P < 0.01$ vs solvent control) were completely attenuated to basal level by addition of RU486 in a dose-dependent manner ($^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$ vs 100 nM Dex without RU486 group, $n = 3$). (C and D) HBPs were treated with 100 nM Dex for 24 h followed by cotreatment with 10 μg/ml actinomycin D to stop RNA synthesis. Total RNA was isolated at the indicated times after addition of actinomycin D and the levels of Ang-1 and VEGF mRNA were determined as described in the legend to Fig. 1. Each point represents the mean Ang-1 (C) and VEGF (D) mRNA level ±SD relative to the 0 h value in logarithmic scale. Ang-1 mRNA was very stable compared to that of VEGF and the stability of Ang-1 and VEGF mRNA was not changed by Dex stimulation ($n = 7$).

expressions by endothelial cells from different tissues can be explained by the heterogeneity of endothelial cells depending on the tissues [29,30].

There are several reports showing glucocorticoid-induced downregulation of VEGF in various cell types including keratinocytes [31], hypoxic brain microvascular endothelial cells [32], chondrocytes [33], and Müller cells [34]. However, the mechanism for Dex-induced downregulation of VEGF mRNA vary depending on the cell type and oxygen status: Dex decreases mRNA stability in cultured keratinocytes and cobalt-treated Müller cells [31,34], whereas Dex does not affect VEGF mRNA stability in chondrocytes and hypoxic porcine BMECs, suggesting an involvement of transcriptional inhibition [32,33]. In our study, Dex did not change mRNA stability of Ang-1 and VEGF, implying transcriptional regulation.

When Dex was routinely administered at doses effective in preventing brain edema, the mean concentrations of Dex in human brain tumor tissues was 225 ng/g, ranging 12–2093 ng/g [35], which is equivalent to 573 nM, ranging 31–5332 nM. These concentrations of Dex in human brain tissues are comparable to those at which Dex increased Ang-1 and decreased VEGF *in vitro* (Figs. 1 and 2). This raises the possibility that Dex may increase Ang-1 and decrease VEGF in HBPs and HBAs in human patients *in vivo* when it is used as an anti-edema drug.

In conclusion, Dex upregulated Ang-1, a strong BBB-stabilizing factor, whereas it downregulated VEGF, a strong permeabilizing factor, in HBAs and HBPs. In addition, Dex failed to significantly influence the levels of VEGF, Ang-1, and Ang-2 in HBMECs. Furthermore, the concentrations of Dex at which it increased Ang-1 and decreased VEGF *in vitro* were comparable to those in brain tissue *in vivo* when Dex is used as anti-edema drug. The Dex-induced increase in Ang-1 and decrease in VEGF may provide a novel mechanism underlying glucocorticoids-induced stabilization of the BBB.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.05.025.

References

[1] P. Ballabh, A. Braun, M. Nedergaard, The blood–brain barrier: an overview: structure, regulation, and clinical implications, Neurobiol. Dis. 16 (2004) 1–13.
[2] R. Daneman, B.A. Barres, The blood–brain barrier—lessons from moody flies, Cell 123 (2005) 9–12.
[3] J.H. Kim, J.H. Kim, J.A. Park, S.W. Lee, W.J. Kim, Y.S. Yu, K.W. Kim, Blood–neural barrier: intercellular communication at glio-vascular interface, J. Biochem. Mol. Biol. 39 (2006) 339–345.
[4] R.K. Jain, Molecular regulation of vessel maturation, Nat. Med. 9 (2003) 685–693.
[5] C.H. Lai, K.H. Kuo, The critical component to establish in vitro BBB model: Pericyte, Brain Res. Brain Res. Rev. 50 (2005) 258–265.
[6] E.C. Kaal, C.J. Vecht, The management of brain edema in brain tumors, Curr. Opin. Oncol. 16 (2004) 593–600.
[7] S.W. Lee, W.J. Kim, Y.K. Choi, H.S. Song, M.J. Son, I.H. Gelman, Y.J. Kim, K.W. Kim, SSeCKS regulates angiogenesis and tight junction formation in blood–brain barrier, Nat. Med. 9 (2003) 900–906.
[8] S. Hori, S. Ohtsuki, K. Hosoya, E. Nakashima, T. Terasaki, A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro, J. Neurochem. 89 (2004) 503–513.
[9] S. Nag, T. Papneja, R. Venugopalan, D.J. Stewart, Increased angiopoietin2 expression is associated with endothelial apoptosis and blood–brain barrier breakdown, Lab. Invest. 85 (2005) 1189–1198.
[10] D.R. Senger, S.J. Galli, A.M. Dvorak, C.A. Perruzzi, V.S. Harvey, H.F. Dvorak, Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid, Science 219 (1983) 983–985.
[11] S.M. Weis, D.A. Cheresh, Pathophysiological consequences of VEGF-induced vascular permeability, Nature 437 (2005) 497–504.
[12] D.J. Dumont, T.P. Yamaguchi, R.A. Conlon, J. Rossant, M.L. Breitman, tek, a novel tyrosine kinase gene located on mouse chromosome 4, is expressed in endothelial cells and their presumptive precursors, Oncogene 7 (1992) 1471–1480.
[13] A. Iwama, I. Hamaguchi, M. Hashiyama, Y. Murayama, K. Yasunaga, T. Suda, Molecular cloning and characterization of mouse TIE and TEK receptor tyrosine kinase genes and their expression in hematopoietic stem cells, Biochem. Biophys. Res. Commun. 195 (1993) 301–309.
[14] G.D. Yancopoulos, S. Davis, N.W. Gale, J.S. Rudge, S.J. Wiegand, J. Holash, Vascular-specific growth factors and blood vessel formation, Nature 407 (2000) 242–248.
[15] P.C. Maisonpierre, C. Suri, P.F. Jones, S. Bartunkova, S.J. Wiegand, C. Radziejewski, D. Compton, J. McClain, T.H. Aldrich, N. Papadopoulos, T.J. Daly, S. Davis, T.N. Sato, G.D. Yancopoulos, Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis, Science 277 (1997) 55–60.
[16] G. Thurston, C. Suri, K. Smith, J. McClain, T.N. Sato, G.D. Yancopoulos, D.M. McDonald, Leakage-resistant blood vessels in mice transgenically overexpressing angiopoietin-1, Science 286 (1999) 2511–2514.
[17] A.L. Betz, H.C. Coester, Effect of steroids on edema and sodium uptake of the brain during focal ischemia in rats, Stroke 21 (1990) 1199–1204.
[18] H. Kim, H. Suh, S.A. Jo, H.W. Kim, J.M. Lee, E.H. Kim, Y. Reinwald, S.H. Park, B.H. Min, I. Jo, In vivo bone formation by human marrow stromal cells in biodegradable scaffolds that release dexamethasone and ascorbate-2-phosphate, Biochem. Biophys. Res. Commun. 332 (2005) 1053–1060.
[19] R. Newton, Molecular mechanisms of glucocorticoid action: what is important?, Thorax 55 (2000) 603–613
[20] M. Hangai, T. Murata, N. Miyawaki, C. Spee, J.I. Lim, S. He, D.R. Hinton, S.J. Ryan, Angiopoietin-1 upregulation by vascular endothelial growth factor in human retinal pigment epithelial cells, Invest. Ophthalmol. Vis. Sci. 42 (2001) 1617–1625.
[21] D. Duma, C.M. Jewell, J.A. Cidlowski, Multiple glucocorticoid receptor isoforms and mechanisms of post-translational modification, J. Steroid Biochem. Mol. Biol. 102 (2006) 11–21.
[22] J.G. Tasker, S. Di, R. Malcher-Lopes, Minireview: rapid glucocorticoid signaling via membrane-associated receptors, Endocrinology 147 (2006) 5549–5556.
[23] C. Suri, P.F. Jones, S. Patan, S. Bartunkova, P.C. Maisonpierre, S. Davis, T.N. Sato, G.D. Yancopoulos, Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis, Cell 87 (1996) 1171–1180.
[24] C. Sundberg, M. Kowanetz, L.F. Brown, M. Detmar, H.F. Dvorak, Stable expression of angiopoietin-1 and other markers by cultured pericytes: phenotypic similarities to a subpopulation of cells in maturing vessels during later stages of angiogenesis in vivo, Lab. Invest. 82 (2002) 387–401.
[25] A. Stratmann, W. Risau, K.H. Plate, Cell type-specific expression of angiopoietin-1 and angiopoietin-2 suggests a role in glioblastoma angiogenesis, Am. J. Pathol. 153 (1998) 1459–1466.
[26] Y.S. Park, N.H. Kim, I. Jo, Hypoxia and vascular endothelial growth factor acutely up-regulate angiopoietin-1 and Tie2 mRNA in bovine retinal pericytes, Microvasc. Res. 65 (2003) 125–131.
[27] S. Nag, N. Nourhaghighi, R. Venugopalan, S.L. Asa, D.J. Stewart, Angiopoietins are expressed in the normal rat pituitary gland, Endocr. Pathol. 16 (2005) 67–73.
[28] M. Hangai, S. He, S. Hoffmann, J.I. Lim, S.J. Ryan, D.R. Hinton, Sequential induction of angiogenic growth factors by TNF-alpha in choroidal endothelial cells, J. Neuroimmunol. 171 (2006) 45–56.
[29] W.C. Aird, Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms, Circ. Res. 100 (2007) 158–173.
[30] W.C. Aird, Phenotypic heterogeneity of the endothelium: II. Representative vascular beds, Circ. Res. 100 (2007) 174–190.
[31] J. Gille, K. Reisinger, B. Westphal-Varghese, R. Kaufmann, Decreased mRNA stability as a mechanism of glucocorticoid-mediated inhibition of vascular endothelial growth factor gene expression by cultured keratinocytes, J. Invest. Dermatol. 117 (2001) 1581–1587.
[32] S. Fischer, D. Renz, W. Schaper, G.F. Karliczek, In vitro effects of dexamethasone on hypoxia-induced hyperpermeability and expression of vascular endothelial growth factor, Eur. J. Pharmacol. 411 (2001) 231–243.
[33] J.A. Koedam, J.J. Smink, S.C. van Buul-Offers, Glucocorticoids inhibit vascular endothelial growth factor expression in growth plate chondrocytes, Mol. Cell. Endocrinol. 197 (2002) 35–44.
[34] J.E. Sears, G. Hoppe, Triamcinolone acetonide destabilizes VEGF mRNA in Muller cells under continuous cobalt stimulation, Invest. Ophthalmol. Vis. Sci. 46 (2005) 4336–4341.
[35] U. Nestler, M. Winking, D.K. Boker, The tissue level of dexamethasone in human brain tumors is about 1000 times lower than the cytotoxic concentration in cell culture, Neurol. Res. 24 (2002) 479–482.