Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 91 (2004) 219–224
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

The effects of betamethasone (BM) on endothelial nitric oxide synthase (eNOS) expression in adult baboon femoral arterial endothelial cells

Keiko Aida[a], Qiang Shi[b], Jian Wang[c], John L. VandeBerg[b], Thomas McDonald[a], Peter Nathanielsz[a], Xing Li Wang[c,*]

[a] *Center of Women's Health, New York University, New York, USA*
[b] *Department of Genetics, Southwest Foundation for Biomedical Research and Southwest National Primate Center, San Antonio, TX, USA*
[c] *Divison of Cardiothoracic Surgery, Michael E. DeBakey Department of Surgery, MS Nab 2010, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA*

Received 17 March 2004; accepted 21 May 2004

Abstract

Glucocorticoids have significant effects on endothelium mediated vascular function throughout life. The baboon model has been used extensively to study cellular responses to glucocorticoids at several stages of the lifespan. Endothelial nitric oxide synthase (eNOS) is a major regulator of endothelium dependent arterial vasodilation. We have previously demonstrated that synthetic glucocorticoids down regulate eNOS in the baboon placenta. We have now conducted studies to determine whether glucocorticoids would alter eNOS expression in adult systemic vascular endothelial cells in this important animal model. We explored this potential mechanism in endothelial cells from femoral arteries of adult baboons at necropsy and cultured to the fourth passage. Endothelial cells were treated with 10–100 nM betamethasone for 24 h at 37 °C. Vascular endothelial growth factor (VEGF) was used as a positive control and medium as negative controls. The role of glucocorticoid receptor mediation in betamethasone-induced eNOS changes was investigated with the glucocorticoid receptor antagonist mifepristone. RNA (real-time quantitative RT-PCR) and protein (ELISA) were extracted and measured for eNOS. Expression and subcellular distribution of glucocorticoid receptor were detected with fluorescence labeled antibody microscopy. eNOS mRNA and protein in baboon endothelial cells were downregulated 25% by betamethasone treatment. This effect was attenuated by pre-incubation with mifepristone ($P < 0.01$). VEGF upregulated eNOS transcription and translation ($P < 0.001$), medium did not alter eNOS expression. We observed that mifepristone and VEGF increased glucocorticoid receptor cytoplasmic accumulation by fluorescence microscopy. We conclude that betamethasone can downregulate eNOS in cultured baboon endothelial cells via the glucocorticoid receptor pathway.

Keywords: Baboons; Glucocorticoids; Endothelial cells; eNOS; Glucocorticoid receptors

1. Introduction

Glucocorticoids (GC) are central in the hypothalamo–pituitary–adrenocortical axis and have regulatory roles at all stages of the lifespan in nearly all biological systems including the vasculature. Administration of either natural or synthetic GC has clear benefits in many clinical situations. Endogenous GC secreted by the fetal adrenal plays an indispensable role in the maturation of the fetal cardiovascular system [1]. Glucocorticoid administration to the fetal sheep accelerates the peripheral vasoconstrictor responses to endothelin that occur naturally as the fetus matures in late gestation [2,3]. Synthetic glucocorticoids are routinely administered to accelerate fetal lung maturation in pregnant women who are at risk of premature delivery between 24 and 34 weeks gestation. Preterm birth occurs in 10% of all live births and is a major cause of neonatal mortality and morbidity. Antenatal synthetic GC therapy reduces the risk of infant mortality by approximately 30%, the risk of neonatal respiratory distress syndrome by approximately

* Corresponding author. Tel.: +1 713 798 5485; fax: +1 713 798 1705.
E-mail address: xlwang@bcm.tmc.edu (X.L. Wang).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.05.004

50%, and the risk of both intracranial hemorrhage and periventricular leukomalacia by approximately 70% [4].

Despite these clear benefits, exposure of the fetus to inappropriately high levels of GC for the current stage of gestation raises fetal blood pressure and has significant effects on the fetal peripheral vasculature. Fetal blood pressure is elevated by 30% within 24 h of commencing infusion of synthetic GC to fetal sheep [5] and fetal nonhuman primates [6] or following maternal administration in the pregnant sheep. Elevated GC exposure in fetal life may also affect the function of blood vessels in postnatal life [7]. Various fetal vascular regulatory systems have been shown to be affected by GC including NOS [8], endothelin [2] and the renin–angiotensin system [8,9].

Exposure of adult humans to excess GC, either as a result of external administration or endogenous production during disease states, produces hypertension [10]. In one recent study, men aged 23–33 years with hypertension showed several indications of increased activity of the hypothalamo–pituitary–adrenocortical axis such as enhanced GC sensitivity, cortisol secretion and synthetic GC binding to leucocytes. Thus, understanding the effects of GC on the vasculature at all life stages is critically important.

Whilst some studies demonstrated GC could repress expression of eNOS [11], other studies suggest that GC could either downregulate [12] or have no [13,14] effect on eNOS expression in the adult vasculature. While the fetal and adult sheep have been extensively investigated in relation to these effects of GC there are few studies on nonhuman primates that provide related data. In the current study we sought to determine whether GC affect endothelial eNOS expression in adult baboon femoral artery endothelial cells cultured in vitro and to determine whether any effects observed are mediated via the GC receptor (GR).

2. Experimental

2.1. Baboon femoral artery endothelial cell culture

Primary baboon femoral artery endothelial cells (BFAECs) were isolated from four baboons (two males and two females) that were maintained by the Southwest National Primate Research Center at the Southwest Foundation for Biomedical Research in San Antonio. Segments of femoral arteries approximately 1–3 cm in length were collected from baboons by sterile methods during necropsy for research reasons. Baboon femoral artery endothelial cells were harvested no longer than 2 h after the vessels were excised. The cell isolation procedure was based on previous reports with modifications [15]. The artery was placed in a large sterile dish, and the surface was wiped with 2% antibiotic/antimycotic solution (GIBCO-BRL, Grand Island, NY) in PBS. The artery was then gently cannulated at one end with a short blunt needle and flushed with PBS to remove any blood remaining inside the vessel. It was injected with 0.1% collagenase before the other end was cannulated. The closed artery was incubated at 37 °C for 15 min for digestion. After completion of digestion, the vessel was massaged gently, flushed with medium, and the released cells were collected by centrifugation and resuspended in medium. The cells were seeded immediately on 1.0% gelatin coated culture plates. The medium was F-12K supplemented with 20% fetal calf serum (FCS) (GIBCO-BRL), 75 μg/mL EGCS (Sigma, St. Louis, MO), 50 μg/mL heparin, 10 mM HEPES, 2 mM glutamine and antibiotics. Confluent cells were passaged by 0.05% trypsin and versene solution (GIBCO-BRL), and sub-cultured in a three-fold dilution, i.e. 1:3 split sub-culture. For the current study, we only used up to four passages during culture. The endothelial cells were treated with betamethasone (BM) (10, 50 and 100 nM in 70% ethanol and 20 μL BM solution was added to 2 mL culture medium) for 24 h at 37 °C. Vascular endothelial growth factor (VEGF) was applied as a positive control, and 0.7% ethanol (diluent of BM) or culture medium only was used as a negative control. To investigate the role of GR in BM-induced eNOS changes, the GR antagonist mifepristone (RU486, Sigma) in doses of 1, 100 and 1000 nM was added 30 min before betamethasone treatment. All experiments were conducted in triplicate in two separate assays (n = 6). The study was approved by Institutional Animal Care and Use Committee (IACUC) of the Southwest Foundation for Biomedical Research.

2.2. eNOS mRNA quantitation by real time (RT)-PCR

At the end of experiment, total RNA (50 ng), extracted with Trizol (Invitrogen), was reverse transcribed in a 100 μL reaction containing High-Capacity cDNA Archive Kit (Applied Biosystems Cat. No. 4322171), followed by RT-PCR using human eNOS specific primers (Applied Biosystems Assay ID; Hs00167166_ml) which were developed for use with TaqMan Universal PCR master mix (Applied Biosystems Cat. No. 4304437) and target cDNA. We used a human β-actin specific primer as an endogenous control. A fluorescent probe, which contained a Fam reporter molecule attached to the 5′ end and a non fluorescent quencher linked at the 3′ end were used. For each reaction the amount of fluorescence was detected as the function of the quantity of a reporter dye (Fam) that was released. For the relative quantification of gene expression, the comparative threshold cycle (Ct) method was employed as described in User Bulletin 2 for ABI PRISM® 7700 Sequence Detection Systems. Ct shows the PCR cycle at which an increase in reporter fluorescence above a background signal can first be detected (10 times the standard deviation of the baseline). First, endogenous control Ct values were subtracted from the gene of interest Ct values to derive a ΔCt value. The relative expression of the gene of interest was then evaluated using the expression 2-ΔΔCt, where the value for ΔΔCt was obtained by subtracting the ΔCt of the calibrator from each ΔCt, using the mean of the negative control as the calibrator. All samples were assayed

in separate tubes in one run together with the endogenous control.

2.3. eNOS protein quantification by ELISA

Cells were rinsed three times with cold PBS before being lysed with osmotic lysis buffer (10 mM Tris, pH 7.4, 9.3% SDS, 5 mM $MgCl_2$, 50 μg/mL RNase, 50 μg/mL DNase) for 30 min on ice. The total soluble protein concentration in the supernatant was determined by the Bradford method (Bio-Rad, Cat. No. 500-0006). The extracted proteins were diluted to the same concentration for all samples in the ELISA procedures. We used an eNOS ELISA kit (R&D systems, Cat. No. DEN00). This assay employs the quantitative enzyme immunoassay technique in which a monoclonal antibody specific for human eNOS has been pre-coated onto a microplate. We have used this method to measure eNOS protein in the baboon arterial wall [16]. Standards and samples were pipetted into the wells and any eNOS present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human eNOS was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of eNOS bound in the initial step. The color development was stopped after 10 min and the intensity of the color was measured. The eNOS concentration of each sample was calculated from a standard curve.

2.4. Immunofluorescence identification of intracellular GR distribution

To explore the intracellular distribution of GR under different treatments, 200 μL of endothelial cell suspension was seeded on Lab-Tek culture chambers (Nunc, NY) coated with 1.0% gelatin before they were treated with various conditions described above. At the end of 24 h of treatment, cells were fixed in 2% formalin in PBS at room temperature for 20 min. After they were air-dried, cells were blocked with

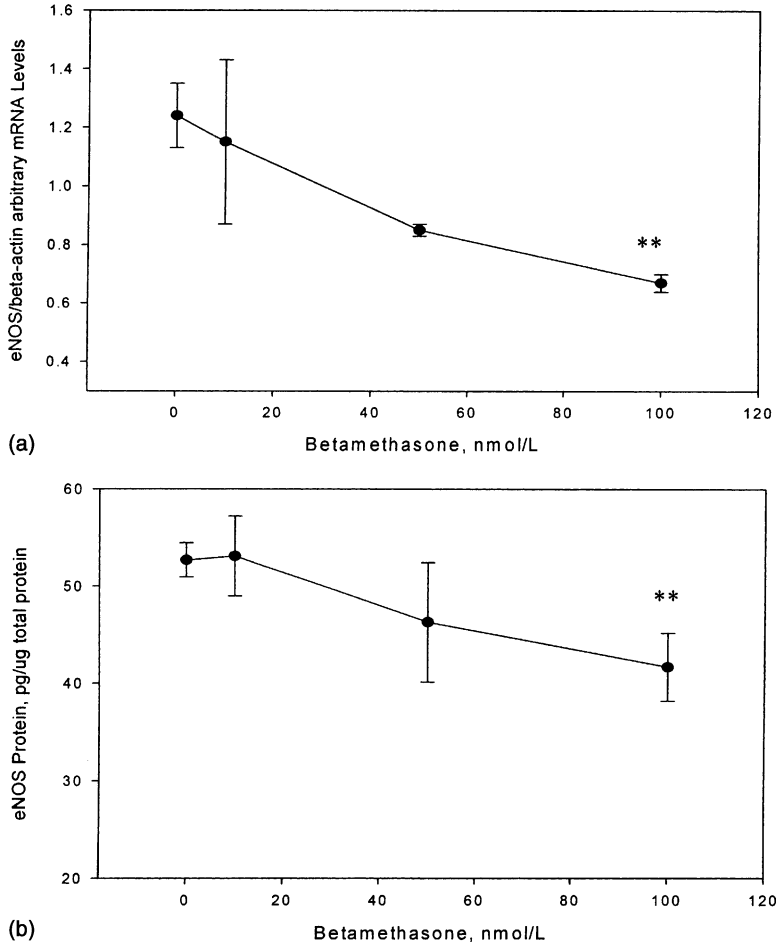

Fig. 1. Dose-dependent changes in eNOS mRNA and protein levels when baboon femoral endothelial cells were treated with BM. (a) Represents relative eNOS mRNA changes after adjusted by the house-keeping β-actin mRNA levels; $^{**}P < 0.01$ by ANOVA test. mRNA levels were measured by real-time quantitative RT-PCR using assay-on-demand (ABI). Primers and probes are designed based on conservative regions of human eNOS and β-actin. (b) Represents changes in eNOS protein levels relative to the total cellular protein in the extracts; $^{**}P < 0.01$ by ANOVA test.

Table 1
Mean ± S.E.M. eNOS protein (pg/μg of total protein, $n = 6$) and mRNA levels (arbitrary units adjusted by β-actin mRNA levels) in baboon endothelial cells treated with different conditions

	Control medium	VEGF 10 ng/mL	Ethanol 0.7%	BM 100 nM	RU 1 μM	BM 100 nM; RU 1 μM
Protein	52.7 ± 1.8	73.2 ± 3.8***	56.6 ± 6.3	41.7 ± 3.5*	55.3 ± 3.1	56.9 ± 1.3
mRNA	1.24 ± 0.11	2.53 ± 0.17***	1.18 ± 0.13	0.67 ± 0.03***	1.30 ± 0.03	1.38 ± 0.07

RU: mifepristone; BM: betamethasone. ***$P < 0.001$, *$P < 0.05$ compared with control cells treated with culture medium only.

10% normal serum from the same species in which the secondary antibody was raised. Cells were then incubated with primary antibodies at 4 °C overnight. Anti-human GR (Alexis Corp, San Diego, CA) at a dilution of 1:400 was used to detect GR in BFAECs. The primary antibody was detected by FITC-labeled secondary antibody (Santa Cruz, CA) and images were taken under a NIKON Eclipse E800 microscope. Nuclei were stained blue with 4′6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma, Cat. No. 32670). The fluorescence reaction was allowed to progress in complete darkness.

2.5. Statistical analysis

Results are presented as mean ± S.E.M. and compared by Student's *t*-test for between groups and by univariate ANOVA for three or more groups. Two-tailed $P < 0.05$ was regarded as statistically significant.

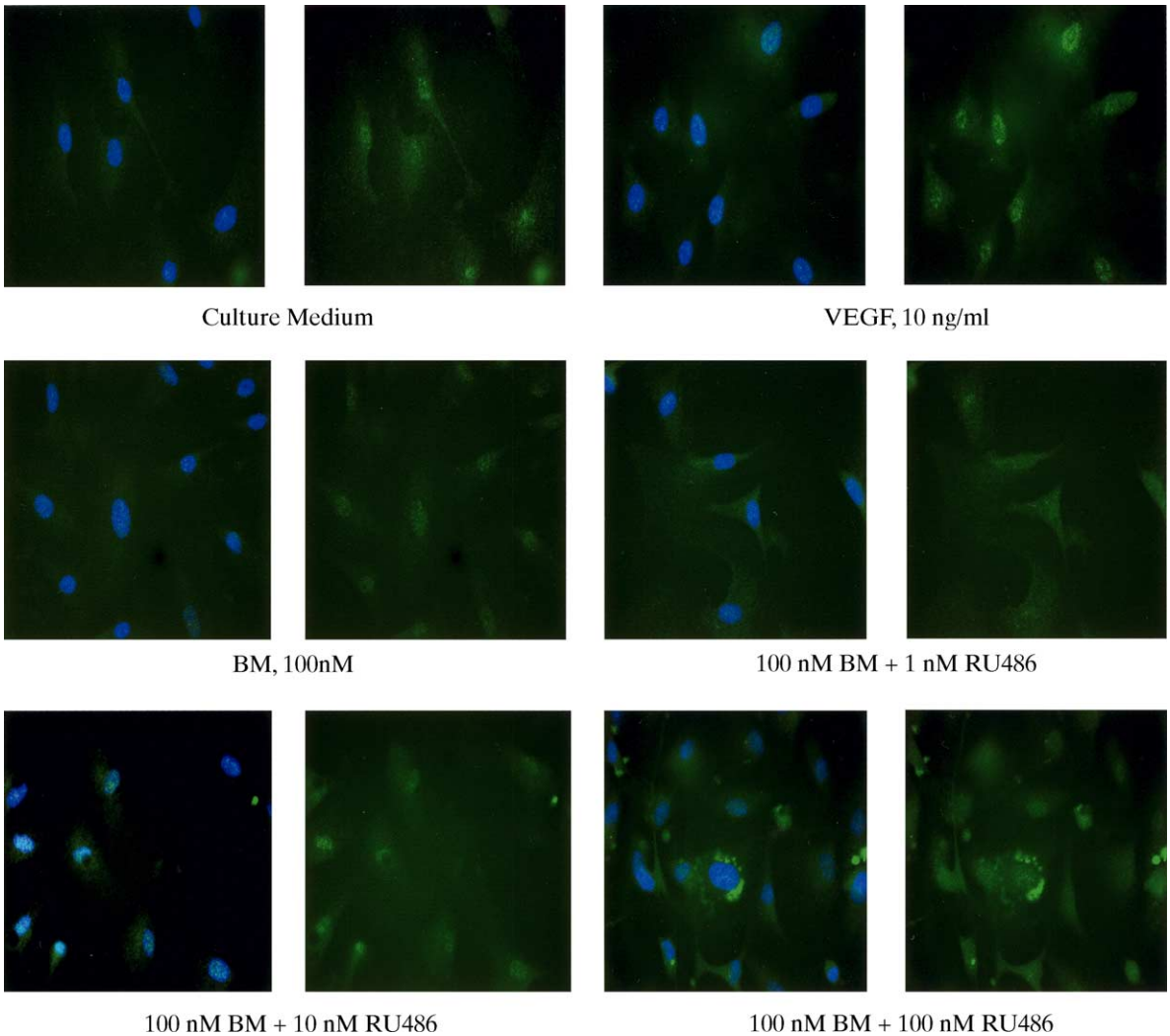

Fig. 2. Fluorescence microscopic views of changes in GR staining using FITC-labeled anti-human GR antibody (stained green color). Cells treated under different conditions as described in Experimental Section 2.4 were viewed using a fluorescence microscope (400×). Nuclei were stained with DAPI as blue fluorescence color. For each treatment condition, left side picture is the composite of green GR staining and DAPI nuclear staining; the right side picture is the green GR staining only in order to demonstrate more clearly the nuclear GR distribution.

3. Results

3.1. Effects of betamethasone on eNOS expression

There was a dose-dependent decline in eNOS expression with increasing doses of BM (Fig. 1, $P < 0.01$). This decrease was much more dramatic in eNOS mRNA (Fig. 1a, from 1.24 $\pm$ 0.11 to 0.67 $\pm$ 0.03, representing a 46% reduction, $P < 0.01$) than in protein levels (Fig. 1b, from 52.7 $\pm$ 1.7 to 41.7 $\pm$ 3.5 pg/μg total proteins, representing a 21% reduction, $P < 0.01$). As a positive control, 10 ng/mL VEGF significantly increased eNOS protein (38% increase) and mRNA (two-fold increase) levels, whereas the vehicle ethanol at the final concentration of 0.7% had no effect on eNOS protein and mRNA levels (Table 1).

3.2. Role of GR in BM-induced eNOS depression

In order to investigate the mechanisms of BM-induced eNOS depression, we pre-incubated the baboon endothelial cells with the GR antagonist mifepristone (RU486). Whilst RU486 had no effect by itself on eNOS expression, it did attenuate the inhibiting effect of BM on eNOS (Table 1, $P < 0.01$). This effect appeared to be at the transcriptional level since the same degree of restoration of expression was also observed at eNOS mRNA level. We further examined the expression and distribution of GR receptors. As shown in Fig. 2, there was a low level of GR expression in nuclei as well as cytoplasm of control cells. VEGF treatment appeared to increase GR expression as shown by increased levels in both nuclei and cytoplasm in a diffuse form. Treatment with BM appeared to have little effect on GR expression, but RU486 caused more GR diffuse into the cytoplasm. At the high RU486 concentration (100 nM), the cytoplasmic GR clustered around the perinuclear region. However, the nuclear content of GR did not appear to change, especially when compared with the fluorescence intensity of GR specific staining in the nuclei of VEGF treated endothelial cells. However, it should be noted that these conclusions are not quantitatively based but mainly derived from visual observations.

4. Discussion

As discussed in the introduction both the sheep and the baboon have been used extensively to follow the effects of GC on the vasculature at different stages of life. We have shown that exposure of the fetal sheep and baboon to synthetic glucocorticoids alters endothelium dependent responses of small peripheral vessels [2,17]. With the wide spread use of GC in various clinical conditions, it is important to understand the potential side effects that are caused by GC administration at all stages of development and postnatal life. Results of our previous study with baboon fetal placental vessels demonstrated a decreased eNOS expression and enzyme activity [18,19]. This decrease in placental vascular endothelial eNOS occurs in the presence of increased fetal blood pressure, [6] a situation that should increase eNOS as a response to increased sheer stress on the vascular endothelium.

In the present study using adult baboon femoral artery endothelial cells, we have shown the same depressive effect on eNOS mRNA and protein levels. This effect is BM dose-dependent. Similar to our previous in vivo data, [18] the decrease of mRNA levels was much more dramatic than of protein levels. This observation is consistent with the mechanism of action being reduced transcription rather than translation.

Our current results suggest that this inhibiting effect may be mediated through the GR receptor pathway since we have further shown that the effect on eNOS expression is largely attenuated when endothelial cells are preconditioned by RU486. GR is a nuclear receptor which mediates the genomic responses to the hormones [20]. When cells are exposed to steroid hormones, such as BM, GR binds with BM and translocates to nucleus to fulfill its transcriptional function [21]. Short-term incubation with BM (5 h) may cause a decrease in GR level [22]. However, after prolonged incubation (24 h), this effect may be diminished [21]. This result is consistent with our observation of little effect on GR expression 24 h after BM treatment.

Our experiments have further shown that cells treated with GR inhibitor RU486 in presence of 100 nM BM had increased cytoplasmic GR concentrations (Fig. 2). RU486 is a dual antagonist with both antiprogesterone and antiglucocorticosteriod function. Its antiglucocorticosteriod effect is likely to be mediated through the formation of a heterooligomeric complex with GR, which impedes or slows down the formation of the activated receptor form observed after binding an agonist [20]. Therefore, more RU486–GR complexes are present in cytoplasm, and GR function is inhibited. As shown in Fig. 2, at a high concentration of RU486, clustered accumulation of GR was observed in the perinuclear location as compared to cytoplasmic and nuclear distribution at lower RU486 concentrations. This may be inconsistent with the observation by Schaaf and Cidlowski in which they observed both BM and RU486 caused nucleus translocation of GR [23]. Whilst it is still under investigation, the cell type specificity and co-treatment of BM and RU486 could be the reason for the cytoplasmic sequestration in our experiment. We also note the significant nuclear GR staining in our control experiment, which could be due to the presence of glucocorticoids (<15.6 nM) in fetal calf serum used in our culture medium [24].

Whilst it is known that glucocorticoids reduce VEGF expression, [22,25–28] we, for the first time, show that VEGF may upregulate GR expression, especially nuclear GR concentrations (Fig. 2). At the same time, VEGF, as a positive control, has also upregulated eNOS expression. It is not clear, however, whether the upregulated GR expression by VEGF is associated with high or low GR function. If the function of GR is upregulated by VEGF, it would then be difficult to reconcile the inhibiting effect by GR agonist, such as BM,

on eNOS expression as observed by us and Wallerath et al. [12]. It is tempting to propose that VEGF may act similarly as RU486. Although the GR levels may appear to be high when exposed to VEGF, the GR function may be inhibited. However, more quantitative investigations will be needed in order to confirm the findings.

In summary, we confirm that BM downregulated eNOS, as observed in vivo in pregnant baboons. Thus, GC appear to have similar effects on the vascular endothelium in both fetal and adult life. This downregulation is mediated via the GR pathway as shown by the attenuating effect of the RU486 treatment. Our findings of increased GR accumulation when cells were treated by VEGF, in a similar pattern to those treated by RU486, is intriguing and deserves more investigation.

Acknowledgement

The study is supported by NIH grants R01HL66053, RO1 HD 21350 and P51 RR013986.

References

[1] N. Unno, et al., Blood pressure and heart rate in the ovine fetus: ontogenic changes and effects of fetal adrenalectomy, Am. J. Physiol. 276 (1999) H248–H256.
[2] C.C. Docherty, J. Kalmar-Nagy, M. Engelen, S.V. Koenen, M. Nijland, R.E. Kuc, A.P. Davenport, P.W. Nathanielsz, Effect of in vivo fetal infusion of dexamethasone at 0.75 GA on fetal ovine resistance artery responses to ET-1, Am. J. Physiol. Regul. Integr. Comp. Physiol. 281 (2001) R261–R268.
[3] C.C. Docherty, J. Kalmar-Nagy, M. Engelen, P.W. Nathanielsz, Development of fetal vascular responses to endothelin-1 and acetylcholine in the sheep, Am. J. Physiol. Regul. Integr. Comp. Physiol. 280 (2001) R554–R562.
[4] NIH, NIH Consensus Development Panel on the effect of corticosteroids for fetal maturation on perinatal outcomes, effect of corticosteroids for fetal maturation on perinatal outcomes, JAMA 273 (1995) 413–418.
[5] J.B. Derks, D.A. Giussani, S.L. Jenkins, R.A. Wentworth, G.H. Visser, J.F. Padbury, P.W. Nathanielsz, A comparative study of cardiovascular, endocrine and behavioural effects of betamethasone and dexamethasone administration to fetal sheep, J. Physiol. 499 (1997) 217–226.
[6] S.V. Koenen, C.A. Mecenas, G.S. Smith, S. Jenkins, P.W. Nathanielsz, Effects of maternal betamethasone administration on fetal and maternal blood pressure and heart rate in the baboon at 0.7 of gestation, Am. J. Obstet. Gynecol. 186 (2002) 812–817.
[7] J. Molnar, M.J. Nijland, D.C. Howe, P.W. Nathanielsz, Evidence for microvascular dysfunction after prenatal dexamethasone at 0.7, 0.75, and 0.8 gestation in sheep, Am. J. Physiol. Regul. Integr. Comp. Physiol. 283 (2002) R561–R567.
[8] R.S. Fishel, S. Eisenberg, S.Y. Shai, R.A. Redden, K.E. Bernstein, B.C. Berk, Glucocorticoids induce angiotensin-converting enzyme expression in vascular smooth muscle, Hypertension 25 (1995) 343–349.
[9] A.J. Forhead, C.E. Gillespie, A.L. Fowden, Role of cortisol in the ontogenic control of pulmonary and renal angiotensin-converting enzyme in fetal sheep near term, J. Physiol. 526 (2000) 409–416.
[10] J.A. Whitworth, M.A. Brown, J.J. Kelly, P.M. Williamson, Mechanisms of cortisol-induced hypertension in humans, Steroids 60 (1995) 76–80.
[11] D.G. Johns, A.M. Dorrance, N.L. Tramontini, R.C. Webb, Glucocorticoids inhibit tetrahydrobiopterin-dependent endothelial function, Exp. Biol. Med. (Maywood) 226 (2001) 27–31.
[12] T. Wallerath, et al., Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 13357–13362.
[13] B.O. Okoye, P.D. Losty, M.J. Fisher, I. Wilmott, D.A. Lloyd, Effect of dexamethasone on endothelial nitric oxide synthase in experimental congenital diaphragmatic hernia, Arch. Dis. Child Fetal Neonatal Ed. 78 (1998) F204–F208.
[14] M.W. Radomski, R.M. Palmer, S. Moncada, Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 10043–10047.
[15] Q. Shi, J. Wang, X.L. Wang, J.L. VandeBerg, Comparative analysis of vascular endothelial cell activation by TNF-alpha and LPS in humans and baboons, Cell. Biochem. Biophysics 40 (2004) 289–303.
[16] J. Wang, D. Felux, J. VandeBerg, X.L. Wang, Discordance of endothelial nitric oxide synthase in the arterial wall and its circulating products in baboons: interactions with redox metabolism, Eur. J. Clin. Invest. 33 (2003) 288–295.
[17] J. Molnar, D.C. Howe, M.J. Nijland, P.W. Nathanielsz, Prenatal dexamethasone leads to both endothelial dysfunction and vasodilatory compensation in sheep, J. Physiol. 547 (2003) 61–66.
[18] K. Aida, N. Schlabritz-Lutsevich, T.J. McDonald, X.L. Wang, J. Wang, M. Raveedran, P.W. Nathanielsz, The effects of betamethasone (bM) administration to the pregnant baboon at 0.7 gestation on eNOS mRNA expression in the placenta, J. Soc. Gynecol. Investig. 10 (2003) 155A.
[19] K. Aida, N. Schlabritz-Lutsevich, T.J. McDonald, X.L. Wang, J. Wang, M. Raveedran, P.W. Nathanielsz, Antenatal betamethasone administration to the pregnant baboon inhibits fetal ventricular iNOS mRNA, J. Soc. Gynecol. Investig. 10 (2003) 343A.
[20] F. Cadepond, A. Ulmann, E.E. Baulieu, RU486 (mifepristone): mechanisms of action and clinical uses, Annu. Rev. Med. 48 (1997) 129–156.
[21] M. Nishi, N. Takenaka, N. Morita, T. Ito, H. Ozawa, M. Kawata, Real-time imaging of glucocorticoid receptor dynamics in living neurons and glial cells in comparison with non-neural cells, Eur. J. Neurosci. 11 (1999) 1927–1936.
[22] D.L. Bellingham, M. Sar, J.A. Cidlowski, Ligand-dependent downregulation of stably transfected human glucocorticoid receptors is associated with the loss of functional glucocorticoid responsiveness, Mol. Endocrinol. 6 (1992) 2090–2102.
[23] M.J. Schaaf, J.A. Cidlowski, Molecular determinants of glucocorticoid receptor mobility in living cells: the importance of ligand affinity, Mol. Cell. Biol. 23 (2003) 1922–1934.
[24] T. Raivio, J.J. Palvimo, S. Kannisto, R. Voutilainen, O.A. Janne, Transactivation assay for determination of glucocorticoid bioactivity in human serum, J. Clin. Endocrinol. Metab. 87 (2002) 3740–3744.
[25] U.B. Kompella, N. Bandi, S.P. Ayalasomayajula, Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression, Invest. Ophthalmol. Vis. Sci. 44 (2003) 1192–1201.
[26] 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.
[27] I.S. Ha, E.Y. Um, H.R. Jung, H.W. Park, H.I. Cheong, Y. Choi, Glucocorticoid diminishes vascular endothelial growth factor and exacerbates proteinuria in rats with mesangial proliferative glomerulonephritis, Am. J. Kidney Dis. 39 (2002) 1001–1010.
[28] I.A. Halaby, et al., Glucocorticoid-regulated VEGF expression in ischemic skeletal muscle, Mol. Ther. 5 (2002) 300–306.