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Nitric oxide and hypoxia stimulate erythropoietin receptor via MAPK kinase in endothelial cells

Bojana B. Beleslin Cokic [a,*], Vladan P. Cokic [b], Sukanya Suresh [c], Stacey Wirt [c], Constance Tom Noguchi [c]

[a] Clinic for Endocrinology, Diabetes and Metabolic Diseases, Genetic Laboratory, Clinical Center of Serbia, Belgrade, Serbia
[b] Laboratory of Experimental Hematology, Institute for Medical Research, University of Belgrade, Belgrade, Serbia;
[c] Molecular Medicine Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA

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ABSTRACT

Erythropoietin receptor (EPOR) expression level determines the extent of erythropoietin (EPO) response. Previously we showed that EPOR expression in endothelial cells is increased at low oxygen tension and that EPO stimulation of endothelial cells during hypoxia can increase endothelial nitric oxide (NO) synthase (eNOS) expression and activation as well as NO production. We now observe that while EPO can stimulate NO production, NO in turn can regulate EPOR expression. Human umbilical vein endothelial cells (HUVEC) treated with 10–50 μM of NO donor diethylenetriamine NONOate (DETANO) for 24 h showed significant induction of EPOR gene expression at 5% and 2% of oxygen. Also human bone marrow microvascular endothelial cell line (TrHBMEC) cultured at 21 and 2% oxygen with 50 μM DETANO demonstrated a time and oxygen dependent induction of EPOR mRNA expression after 24 and 48 h, particularly at low oxygen tension. EPOR protein was also induced by DETANO at 2% oxygen in TrHBMEC and HUVEC. The activation of signaling pathways by NO donor stimulation appeared to be distinct from EPO stimulation. In reporter gene assays, DETANO treatment of HeLa cells at 2% oxygen increased EPOR promoter activity indicated by a 48% increase in luciferase activity with a 2 kb EPOR promoter fragment and a 71% increase in activity with a minimal EPOR promoter fragment containing 0.2 kb 5′. We found that DETANO activated MAPK kinase in TrHBMEC both in normoxia and hypoxia, while MAPK kinase inhibition showed significant reduction of EPOR mRNA gene expression at low oxygen tension, suggesting MAPK involvement in NO mediated induction of EPOR. Furthermore, DETANO stimulated Akt anti-apoptotic activity after 30 min in normoxia, whereas it inhibited Akt phosphorylation in hypoxia. In contrast, EPO did not significantly increase MAPK activity while EPO stimulated Akt phosphorylation in TrHBMEC in normoxia and hypoxia. These observations provide a new effect of NO on EPOR expression to enhance EPO response in endothelial cells, particularly at low oxygen tensions.

Introduction

Erythropoietin (EPO), expressed in renal peritubular cells, is secreted into the circulation and targets EPO receptor (EPOR) expressing erythroid progenitor cells in the bone marrow. EPO binding to EPOR on erythroid progenitor cells activates Janus tyrosine kinase 2 (JAK2)/signal transducer and activator of transcription 5 (STAT5), phosphatidylinositol-3 kinase (PI-3K)/Akt, and RAS/mitogen-activated protein kinase (MAPK) signaling pathways. In addition to EPO/EPOR activity in the erythroid system, EPOR is also expressed in other tissues including neuronal cells and brain, endothelial cells and the cardiovascular system, female reproductive organs, and the gut (Noguchi et al., 2008). Similar signal transduction pathways have been observed in response to EPO stimulation in erythroid and non-erythroid tissues. In addition, EPO enhanced NO bioavailability through eNOS transcription and activation (Beleslin-Cokic et al., 2004).

We observed that hypoxia and EPO increased EPOR gene expression and protein level in the vein, artery and microvascular endothelial cells (Beleslin-Cokic et al., 2004, 2011). In addition, EPO dose- and time-dependently stimulated NO production (Beleslin-Cokic et al., 2011). In accordance with NO activation of soluble guanosine cyclase to produce cyclic guanosine monophosphate (cGMP), we observed that EPO induced cGMP production. These results suggested that low oxygen tension increases endothelial cell capacity to produce NO in response to EPO by induction of both EPOR and eNOS (Beleslin-Cokic et al., 2004). Interestingly, in neuronal cells, NO donor increased EPOR expression as well as EPOR promoter activity in a reporter gene assay, which gives rise to protection against hypoxia even in the absence of exogenous Epo (Chen et al., 2010).

It has been demonstrated that NO, as well as hypoxia, elevated serum levels of EPO in ex-hypoxic polycythemic mice, while EPO demonstrated NOS-dependence in stimulation of cGMP levels in hypoxia (Ohigashi et al., 1993). In addition, non specific NOS inhibitor L-NAME inhibited Epo production in anemic mice (Tarumoto et al., 2000). NO

* Corresponding author at: Clinic for Endocrinology, Diabetes and Metabolic Diseases, Genetic laboratory, Clinical Center of Serbia, Belgrade, Serbia, Dr. Subotica 13, 11000 Belgrade, Serbia.
E-mail address: miljuc@yahoo.com (B.B.B. Cokic).

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mediated Epo stimulated increase of the circulating endothelial progenitor cells (EPCs), with endothelial properties. Epo signaling mediated via binding to EPOR induced Akt/eNOS phosphorylation and NO synthesis on EPCs, with an antiapoptotic action, and averted abnormal remodeling of the injured carotid artery (Urao et al., 2006: d'Uscio et al., 2007). eNOS has also a major role in EPO mediated vascular protection; EPO activation of EPOR increased expression of phosphorylated Ser1177-eNOS and normalized the vasodilator reaction to acetylcholine (d'Uscio et al., 2007). Interestingly, the role of hypoxia inducible factor (HIF) in cardiac stress response including hypoxia preconditioning and ischemia-reperfusion injury is associated with the induction of cardioprotective molecules, such as inducible NOS and EPO, that improve myocardial damages (Tekin et al., 2010).

We now examine the contribution of NO on EPOR expression and related signaling pathways in endothelial cells. To determine the relationship of EPOR gene to NO stimulation, NO donor was used to induce mRNA and protein level of EpoR in primary human endothelial cells of vein (HUVEC) and cells from a human bone marrow microvascular endothelial cell (TrHBMEC) cultured at normoxia and at reduced oxygen level, that may be experienced physiologically or pathologically. Reporter gene assays suggested that the increase in EPOR expression by NO donor treatment is mediated in part by stimulated EPOR promoter activity in hypoxia. NO stimulation in hippocampal slices was associated with MAPK activation (Kanterewicz et al., 1998) and we determined that NO also stimulated MAPK signaling pathway both in normoxia and hypoxia in endothelial cells. Also, treating endothelial cells with MAPK inhibitor significantly reduced EPOR mRNA expression after stimulation with NO donor particularly at low oxygen tension. While NO inhibited Akt signaling pathway in hypoxia, we found that EPO triggered anti-apoptotic Akt signaling pathway in normoxia and in early response to hypoxia. Therefore, these signal transduction pathways in response to NO are distinct from EPO response.

Materials and methods

Cell cultures

Human umbilical vein endothelial cells (HUVECs; Clonetics, Walkersville, MD) and transformed human bone marrow endothelial cells (TrHBMECs) were grown as previously described (Beleslin-Cokic et al., 2004). Briefly, HUVECs were cultured in endothelial basal media (EBM-2) containing 2% fetal bovine serum (FBS) and cytokines under 5% of CO_2. TrHBMECs were grown in Dulbecco modified Eagle medium (DMEM) with 10% FBS in 5% CO_2. Human cervical adenocarcinoma (HeLa) cells were cultured in DMEM, containing penicillin–streptomycin, 10% FBS under 5% CO_2 with balanced 95% room air. All cells were cultured in a humidified atmosphere at 37 °C (Forma Scientific, Marietta, OH). Before exposure to reduced oxygen, TrHBMECs were trypsinized, washed and cultured in DMEM medium. HUVECs were washed in HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) buffer and plated in EBM-2 containing 2% FBS and cytokines, and cultured at low oxygen tension (5% or 2% O_2). When confluent, the endothelial cells were treated with 10–50 μM NO donor diethylenetriamine NONOate (DETANO, Alexis Biochemicals, San Diego, CA) and EPO, with or without MAPK inhibitor, PD98059 (Cell Signaling Technology, Danvers, MA), at different oxygen tensions (2, 5 and 21% O_2). DETANO is a NO donor with a half-life of 20 h at 37 °C. Following incubation cells were washed once with PBS or HEPES buffer. For investigating the role of MAPK signaling on NO induction of EPOR, TrHBMECs were cultured in 10% FBS DMEM for 48 h either at 2% or 21% O_2. When cells reached 90% confluence, 50 μM of MAPK inhibitor was added to the culture for one hour, then washed with PBS and cultured in media with 50 μM DETANO for an additional 24 h.

Quantitative real-time RT-polymerase chain reaction analyses

After incubation at different oxygen tensions, endothelial cells were harvested. RNA was isolated using STAT 60 (Tel-TEST, Friendswood, TX) and treated with RNase-Free DNase (Promega, Madison, WI). Total RNA from each sample was used for first-strand cDNA synthesis using reverse transcriptase and oligo $d(T)_{16}$ (Applied Biosystems, Foster City, CA). Quantitative real-time RT-polymerase chain reaction (PCR) analyses were performed using a 7700 or 7900 Sequence Detector and Taqman EPOR oligonucleotide probes and primers (Applied Biosystems) as previously described (Beleslin-Cokic et al., 2004). β-Actin was used as an internal control for the total amount of RNA analyzed.

Protein analysis

Endothelial cells were treated with lysis buffer and scraped from the plate. For measurement of protein concentration in examined samples we used a copper chelation procedure and bicinchoninic acid colorimetric assay (BCA Protein Assay Reagent, Pierce, Rockford, IL). For EPOR analysis, 1 mg protein was incubated with EPOR rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 4 °C overnight. The immunocomplex was captured by adding protein A–Agarose (Santa Cruz Biotechnology), agitated at 4 °C for 2 h, centrifuged, and the

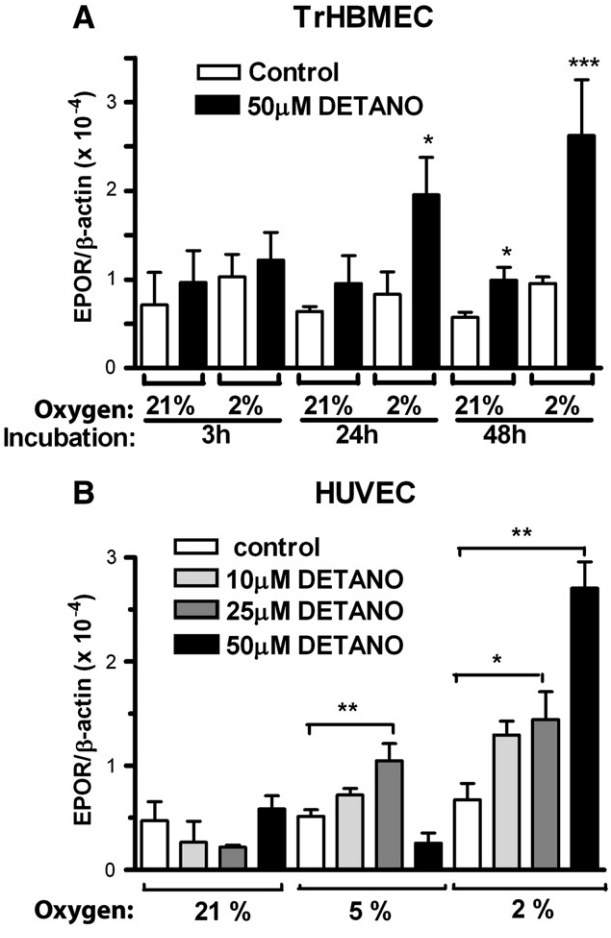

Fig. 1. NO stimulated EPOR mRNA expression in endothelial cells. A) EPOR expression was normalized to β-actin expression in TrHBMEC during treatment with 50 μM of DETANO at 2% and 21% O_2 after 3, 24 and 48 h of incubation. B) EPOR expression was normalized to β-actin expression in HUVEC during treatment with 10, 25 and 50 μM of DETANO at 2%, 5% and 21% O_2 after 24 h of incubation. Values are mean ± SEM (n = 3). *p < 0.05, **p < 0.01 and ***p < 0.001 vs. controls.

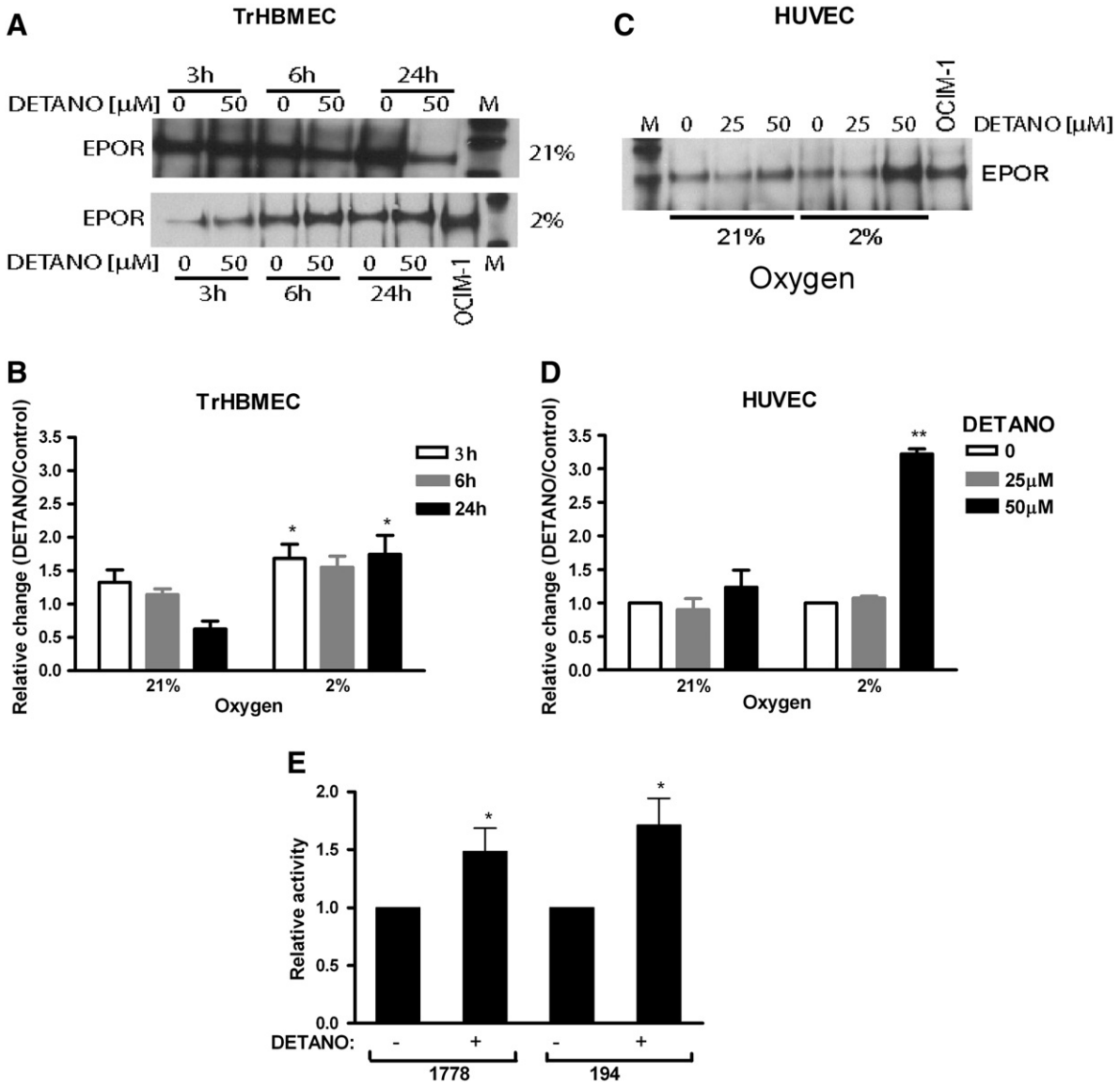

Fig. 2. NO stimulated EPOR protein expression in endothelial cells. A–D) Analysis by Western blotting for EPOR at 2% and 21% of oxygen (A and C) was normalized to control value (B and D) during treatment with 50 μM of DETANO after 3, 6 and 24 h of incubation for TrHBMEC (A and B) and during treatment with 25 and 50 μM of DETANO after 6 h of incubation for HUVEC (C and D). OCIM-1 cell extract was used as positive control. E) Two hEPOR reporter gene constructs (hEPOR −1778 and hEPOR −194) were assessed with and without 50 μM DETANO treatment in HeLa cells at 2% O_2 after 24 h of treatment. Values are mean ± SEM (n = 3). *p < 0.05 and **p < 0.01 vs. controls.

immunoprecipitate washed 2 times with cold PBS. The protein in the sample was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membrane. The membrane was blocked with 5% milk–0.1% Tween 20 for 1 h at room temperature and probed with EPOR rabbit polyclonal antibody overnight at 4 °C. Horseradish peroxide-labeled anti-rabbit immunoglobulin G (IgG) was used as the secondary antibody. Hyperfilm was used to visualize the secondary antibody by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech, Piscataway, NJ). For analysis of MAPK and Akt phosphorylation, cells were washed with cold PBS, left in serum-free media for 6 h, and treated with EPO and DETANO for 15 min, 30 min and 1 h, with or without MAPK inhibitor PD98059 (50 μM). Then, they were lysed and proteins were incubated with phosphoMAPK/MAPK and phosphoAkt/Akt antibody (Cell Signaling Technology) overnight. Cells were washed with HEPES, treated with lysis buffer and scraped from the plate. The proteins were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was probed using as a primary antibody for phosphorylated MAPK and Akt antibody (Cell Signaling Technology) and incubated at 4 °C overnight. The labeled phosphorylated MAPK and Akt was visualized by chemiluminescence. Membrane was then stripped for 1 h at 55 °C, washed, blocked, and incubated with MAPK and Akt antibody overnight at 4 °C and then visualized by chemiluminescence for assessment of total MAPK and Akt.

EPOR reporter gene transfection and activity assay

EPOR reporter gene constructs consisted of the 5′ untranslated transcribed region (UTR) flanking the EPOR coding region and extending to −1778 and −194 bp 5′ of the transcription start site inserted into the pGL3-basic (Promega, Madison, WI) luciferase reporter construct were cotransfected with a control Renilla luciferase construct (pRL-TK) into HeLa cells with Lipofectamine™ 2000 (Invitrogen). Transfected HeLa cells were treated with 50 μM DETANO and incubated at 2% or 21% O_2. After 24 h, cells were harvested, and the activity of the EPOR reporter gene was determined by the Dual Luciferase Assay System (Promega). A promoterless construct containing only the 5′ UTR beginning at +3 of EPOR linked to luciferase was used as a negative control.

Statistical analysis

The one-way analysis of variance (ANOVA) and Dunnett's post tests were applied using Prism 4 software (GraphPad Software Inc., San Diego, CA).

Results

Nitric oxide and hypoxia increase EPOR gene expression in endothelial cells.

We previously reported that hypoxia increased EPOR gene expression in TrHBMEC and lung microvascular endothelial cells (Beleslin-Cokic et al., 2004, 2011). We now show that NO was also able to affect EPOR expression in endothelial cells. DETANO treatment of TrHBMEC resulted in an increase of EPOR expression at 24 to 48 h of incubation depending on oxygen tension (Fig. 1a). At 24 h, DETANO combined with hypoxia stimulated EPOR gene expression in TrHBMEC. After 48 h of treatment, induction of EPOR gene expression by DETANO and hypoxia increased, and was also increased to a lesser extent at normoxia (Fig. 1a). In addition, we demonstrated a dose dependent induction of EPOR gene expression by DETANO in HUVECs both at 2% and 5% O_2 following 24 h of incubation, but not at 21% O_2 (Fig. 1b). Examination of EPOR protein production in endothelial cells revealed that DETANO stimulated EPOR protein expression in hypoxia but not at normoxia in TrHBMEC (Figs. 2a–b). In HUVECs, EPOR protein was also induced by DETANO (by three fold) at 50 μM with hypoxia, but no induction was observed at a lower dose of DETANO at 25 μM (Figs. 2c–d).

EPOR reporter gene assay

We used EPOR promoter reporter genes to determine if NO contributes to the regulation of EPOR expression at the transcription level. The EPOR promoter-luciferase reporter gene construct, containing 2 kb promoter fragment that includes the 5′ UTR and extending upstream

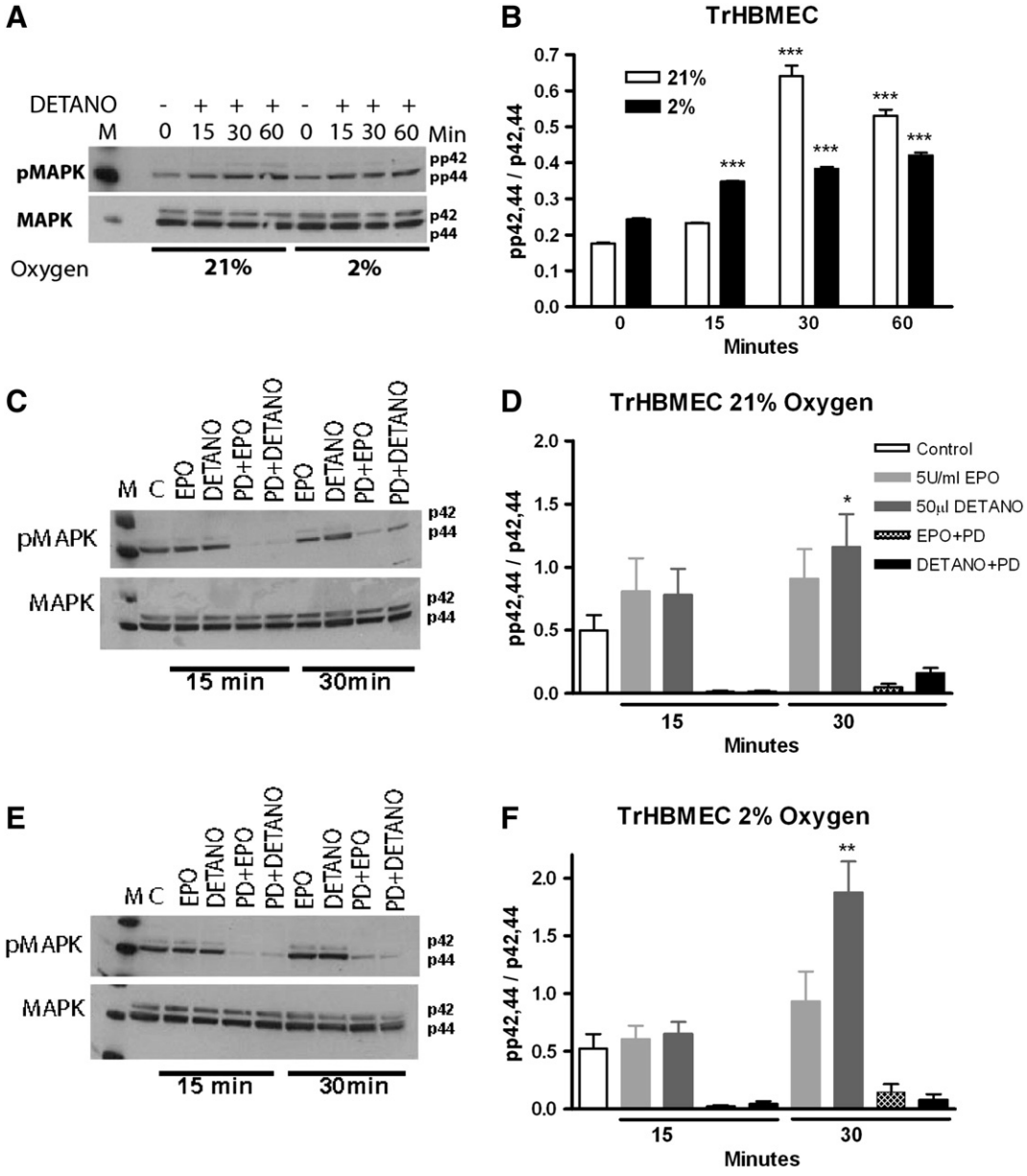

Fig. 3. NO stimulated MAPK phosphorylation (pMAPK) in endothelial cells. A–F) Determination by Western blotting of pMAPK (p42,44) was normalized to total MAPK (p42,44) in TrHBMEC treated with 50 μM of DETANO up to 60 min at 2% and 21% O_2 (A–B) and for treatment without and with EPO (5U/ml), without and with DETANO, and without and with MAPK inhibitor PD98059 (PD) at 21% O_2 (C–D) and at 2% O_2 (E–F). Values are mean ± SEM (n = 3). *p < 0.05, **p < 0.01 vs. controls. ***p < 0.001 vs. samples before treatment.

up to −1778 bp 5′ of the transcription start site, was transfected into HeLa cells. DETANO treated HeLa cells increased luciferase activity 48% at 2% oxygen for the 2 kb EPOR promoter construct after 24 h of incubation at hypoxia (Fig. 2e). Interestingly, truncation of the promoter fragment from −1778 bp 5′ to −198 bp 5′ showed a greater increase of luciferase activity with DETANO treatment by 71%. These results were consistent with the EPOR gene expression data and suggest that NO contributed to the induction of EPOR expression in hypoxia in part at the transcription level. Treatment of the transfected HeLa cells with the NO donor in normoxia did not change the EPOR promoter activity (not shown).

Nitric oxide but not EPO activates MAPK signaling pathway

It has been reported that NO donors augmented the active forms of both p42 MAPK and p44 MAPK (Kanterewicz et al., 1998). DETANO significantly stimulated activation of p42 MAPK and p44 MAPK both in hypoxia and in normoxia in TrHBMECs during the first 60 min (Figs. 3a, b). In contrast, EPO failed to induce significant MAPK phosphorylation in hypoxia and normoxia (Figs. 3c–f). Phosphorylation of p42/44 MAPK in TrHBMECs stimulated with DETANO or EPO was decreased by an order of magnitude or more within 30 min of treatment with the MAPK inhibitor PD98059 at both in hypoxia and normoxia (Figs. 3d, f).

Nitric oxide and hypoxia increase of EPOR gene expression is MAPK dependent

MAPK activation by DETANO (Fig. 3) and the relationship between DETANO and EPOR gene expression raised the possibility that MAPK mediates the change in EPOR expression. Although DETANO did not induce significant EPOR mRNA expression at 21% O_2 in TrHBMEC (Fig. 4a), DETANO treatment almost doubled EPOR gene expression at 2% O_2 after 24 h of incubation (Fig. 4b). This NO donor stimulation of EPOR gene expression was prevented by MAPK inhibitor PD98059. Therefore, NO stimulation of EPOR is partly mediated by MAPK signaling pathway during hypoxia. In contrast, activation of MAPK at normoxia is insufficient to induce EPOR expression.

Nitric oxide inhibits while EPO activates Akt signaling pathway in hypoxia

Akt has downstream anti-apoptotic effects when activated by growth factors through PI-3K. It has been reported that serine/threonine protein kinase Akt mediated the activation of eNOS, leading to increased NO production (Dimmeler et al., 1999). On the other hand, NO donors increased PI-3K regulated Akt phosphorylation and activity in endothelial cells (Kawasaki et al., 2003). EPO also stimulated Akt phosphorylation in normoxia (Akimoto et al., 2000). To compare EPO and DETANO effects on Akt signaling, we found that EPO stimulated Akt phosphorylation within 15 min in normoxia and hypoxia in TrHBMECs (Figs. 5a–b). Moreover, we observed time-dependent effects of EPO during incubation and Akt phosphorylation decreased to baseline levels or lower by 60 min. A time dependent response is also observed with DETANO incubation. DETANO stimulated Akt activity after 30 min in normoxia that decreased toward base line level at 60 min. However, in contrast to EPO, DETANO inhibited Akt phosphorylation in hypoxia throughout the incubation period of 60 min (Figs. 5c–d).

Discussion

We previously described the stimulation of EPOR expression, eNOS activity and elevated NO production by hypoxia and EPO in endothelial cells (Beleslin-Cokic et al., 2004, 2011). Here we show that a NO donor stimulated EPOR gene expression and protein production in TrHBMEC and HUVEC, particularly at reduced oxygen tension. In reporter gene assays, NO donor treatment increased EPOR promoter activity at 2% oxygen. In TrHBMEC, NO donor stimulated activation of p42/p44 MAPK and EPOR gene expression. The induction of EPOR by NO was prevented by MAPK inhibitor. NO donor transiently stimulated Akt activity in normoxia, but inhibited Akt phosphorylation in hypoxia in TrHBMEC. These changes in signal transduction with NO treatment are in contrast to EPO response. EPO did not induce MAPK phosphorylation in hypoxia and normoxia, but stimulated Akt phosphorylation in normoxia and hypoxia in TrHBMEC.

We and others provide increasing evidence of EPO activity in vascular endothelium resulting in NO production. We previously reported that EPO stimulated the phosphorylation of eNOS and increased production of NO and cGMP during hypoxia (Beleslin-Cokic et al., 2004). Moreover, hypoxia and EPO increased EPOR gene expression and protein level, as well as eNOS gene expression (Beleslin-Cokic et al., 2004, 2011). Other studies provided additional evidence of EPO induction of NO. EPO treatment increased NOS activity in endothelial cells of the human coronary artery, pulmonary artery and dermis (Banerjee et al., 2000). Transgenic mice expressing high level of EPO have elevated eNOS protein expression in vascular endothelium and elevated NO levels in the circulation (Ruschitzka et al., 2000). Furthermore, the phosphorylation of eNOS was significantly higher in aorta from EPO transgenic mice than wild-type mice (Su et al., 2012). Transduction of basilar arteries with EPO increased the eNOS protein expression and stimulated eNOS phosphorylation at serine 1177. Basal levels of cGMP were significantly elevated in arteries transduced with EPO, consistent with increased NO production (Santhanam et al., 2006). The observations presented here that NO also stimulates EPOR expression further link NO to EPO activity at hypoxic conditions, likely contributing to endothelial cell stability and microvasculature integrity in maintenance of blood pressure.

Hypoxia has been related to both the upregulation and downregulation of steady-state eNOS mRNA expression. Therefore, it is not unexpected that endothelial cells from various vascular beds react to

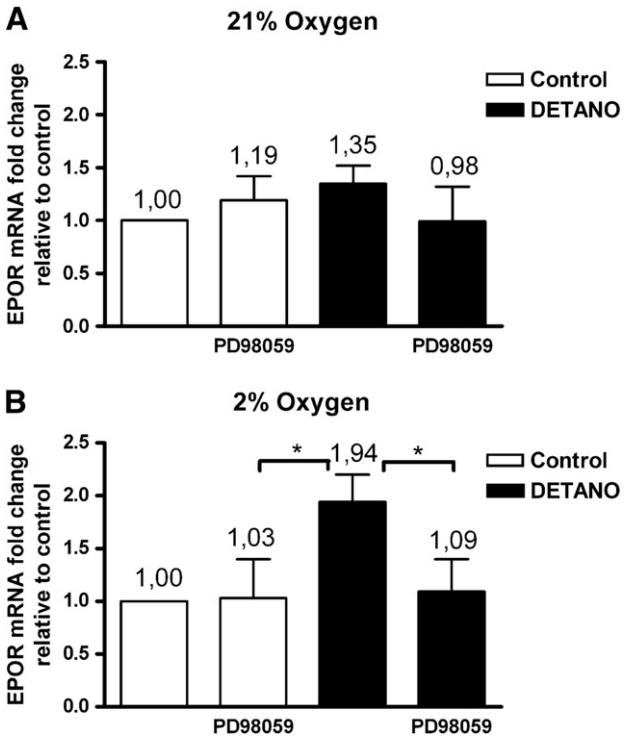

Fig. 4. NO stimulation of EPOR gene expression is MAPK dependent. A–B) Determination of EPOR mRNA expression in TrHBMEC was normalized to β-actin expression during treatment with 50 μM of DETANO without or with 50 μM MAPK inhibitor PD98059 at 21% O_2 (A) and 2% O_2 (B) after 24 h of incubation. Values are mean ± SEM (n = 3). *p < 0.05 and **p < 0.01 vs. controls.

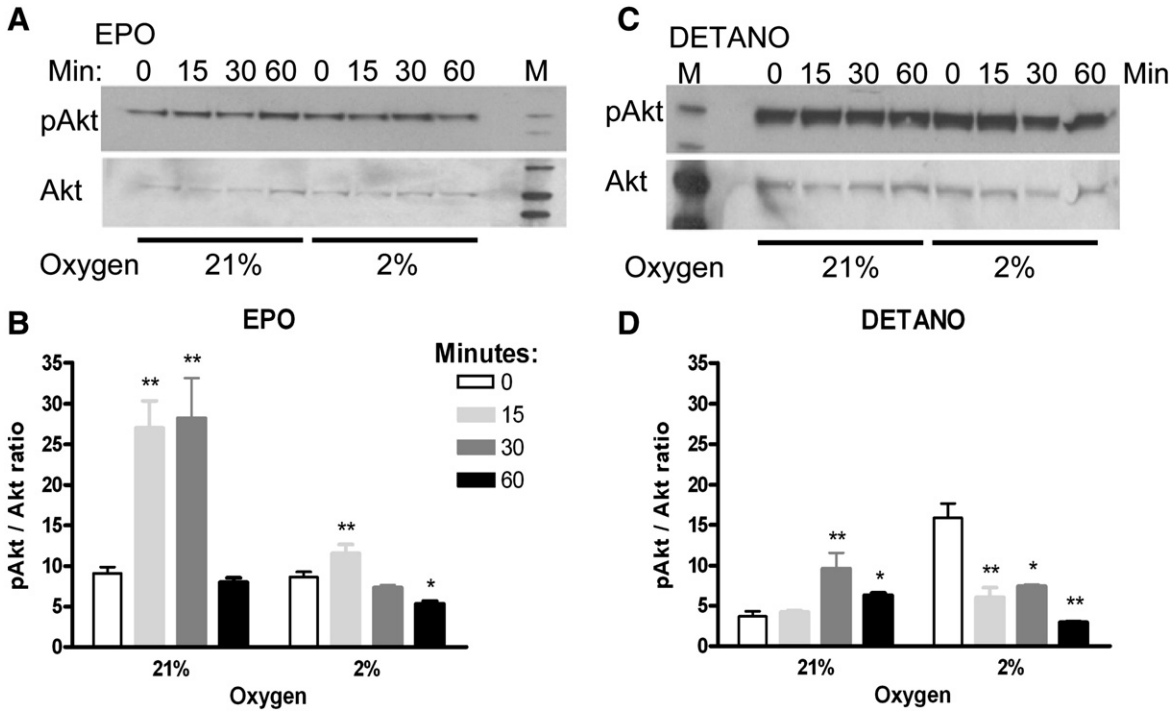

Fig. 5. Phosphorylation of Akt by NO and EPO in endothelial cells. A–D) Determination of pAkt was normalized to total Akt in TrHBMEC during 60 min of treatment with 5U/ml of EPO (A–B) and 50 μM of DETANO (C–D) at 21% and 2% of oxygen. Values are mean ± SEM (n = 3). *p < 0.05 and **p < 0.01vs. untreated cells.

hypoxia in different ways (Tai et al., 2004). Early reports suggested decreased NOS activity in the presence of low oxygen tensions in bovine endothelial cells (Liao et al., 1995; Phelan and Faller, 1996). However, it was subsequently demonstrated that NO generation is enhanced on hypoxia exposure both in bovine (Chen et al., 2008; Presley et al., 2008) and human endothelial cells (Coulet et al., 2003). Moreover, exogenous hypoxia-inducible factor (HIF)-2, as well as hypoxia, markedly increased luciferase reporter activity driven by the eNOS promoter (Arnet et al., 1996; Coulet et al., 2003). In neural cell cultures addition of NO donor to cultures, grown under normoxia and hypoxia, induced EPOR expression. Hypoxia increased NO production as well as EPOR expression, and inhibition of nNOS activity reduced the proportion of EPOR-expressing neurons induced at low oxygen. Conversely, addition of NO donor to cultures grown under normoxia induced EPOR (Chen et al., 2010). In the present study, we demonstrated that in endothelial cells under hypoxic condition NO stimulated EPOR gene expression both at mRNA and protein levels. Furthermore, NO donor increased EPOR promoter activity in a reporter gene assay under hypoxia, but not under normoxia, suggesting that NO regulated EPOR at the transcription level in reduced oxygen conditions. During ischemia or hypoxic stress, NO induction of blood vessel vasodilation would increase delivery of oxygenated blood to hypoxic tissue, while hypoxia induced EPO would promote erythropoiesis to increase the oxygen carrying capacity of blood. The potential of EPO to stimulate NO production by endothelial cells and the apparent feedback of NO to increase EPOR to facilitate an EPO response would further contribute to regulation of vascular tone and facilitate oxygen delivery.

Endogenous NO generation promoted activation of the p42/p44 MAPK. NO affected p42/p44 MAPK by cGMP mediated rapid activation, while late activation was transmitted via inhibition of tyrosine dephosphorylation (Callsen et al., 1998). Here we show that NO stimulated activation of p42/p44 MAPK, and that NO stimulation of EPOR gene expression in hypoxia was MAPK dependent. These data indicate that MAPK activation is necessary but not sufficient for NO induction of EPOR, because NO activates MAPK in both normoxia and hypoxia, but induces EPOR only at hypoxia. Moreover, NO has been reported to markedly decreased Akt activation, while overexpression of constitutively active Akt reduced NO-induced apoptosis (Sang et al., 2011; Wang et al., 2007). The serine/threonine protein kinase Akt can directly phosphorylate eNOS on serine 1179 and activate the enzyme, leading to NO production (Fulton et al., 1999). In the present results, NO stimulated transient Akt activity in normoxia, but inhibited Akt phosphorylation in hypoxia in endothelial cells. Signal transduction response to NO was distinct from EPO response, as EPO stimulated short term Akt phosphorylation in normoxia and hypoxia in TrHBMEC.

Taken together, our presented data suggest that NO response at low oxygen tension results in upregulated EPOR gene expression accompanied by stimulation of the p42/p44 MAPK and inhibition of Akt phosphorylation in endothelial cells. Induction of EPOR by NO could potentially form a positive feedback mechanism for eNOS activation by EPO in endothelial cells at low oxygen tension, while NO inhibited Akt activation in hypoxia. Variation in NO response relating to endothelial cell tissue origin may further contribute to a corresponding variability in EPO response. These results provide further support for an important role of the NO/cGMP system in hypoxic regulation of EPO production, participating in pathogenesis of anemia, repair of injured endothelium, hypertension and ischemia-reperfusion injury.

Acknowledgments

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