Hypoxic induction of vascular endothelial growth factor (VEGF) in human epithelial cells is mediated by increases in mRNA stability**

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Abstract Vessel growth is often associated with ischemia. VEGF, a potent angiogenic factor, has been shown to be induced by low oxygen concentrations. These studies were conducted to investigate the molecular basis of the hypoxia-induced increase in VEGF mRNA. Run-on analysis of VEGF revealed a minimal increase in the rate of gene transcription in a human retinal epithelial cell line grown under hypoxic conditions. Examination of VEGF mRNA stability revealed that the half-life of VEGF transcripts under normoxia was short, 30–45 min, but was dramatically increased to 6–8 h in cells grown under hypoxia. Cobalt chloride, which elevates VEGF and has been suggested to be similar to hypoxia in its mechanism of action, had only a slight effect on decay rate. We postulate that hypoxia-induced increases in mRNA stability provide the sustained increases in VEGF mRNA levels necessary to support a neovascular response.

Key words: Post-transcriptional regulation; Oxygen; Angiogenesis; Cobalt

1. Introduction

Vascular endothelial cell growth factor (VEGF) is a polypeptide that has potent effects on the vascular system, including the ability to induce new vessel growth [1] and increase vascular permeability [2]. Data from a number of laboratories indicate that VEGF can be regulated by local oxygen concentrations in both transformed [3] and normal primary-cultured cells [4]. In addition, we have shown that hypoxia increases VEGF mRNA, protein synthesis, secretion and bioactivity in primary cultures of human pigment epithelial cells (hRPE) [4]. The finding that a vascular growth factor can be regulated by local oxygen concentrations has obvious physiologic implications. Reduced oxygen tension is a characteristic of virtually all circumstances in which new vessel growth is observed. Although solid tumors are highly vascularized, it has been well-documented that the immature architecture of tumor blood vessels, coupled with their high level of permeability, leads to local collapse of tumor vasculature with resulting hypoxia [5]. In addition, cell growth in some tumors such as glioblastomas is so rapid that tumor volume exceeds the perfusion capacity of the vasculature. Similarly, wounds have been shown to have a low oxygen environment, with wound fluid being effectively anoxic [6].

Ischemia, with its concomitant hypoxia, has also been implicated in the development of new blood vessels which are a component of diabetes and retinopathy of prematurity [7,8]. In an experimental model in which a primate retina was rendered ischemic by vein occlusion, there was reproducible development of new blood vessels. We have shown that the levels of VEGF in these animals correlate temporally, spatially and quantitatively with the extent of ocular neovascularization [9]. Moreover, ischemia-associated ocular angiogenesis in humans has been correlated with increased levels of VEGF [10,11].

In an effort to gain further insight into the regulation of VEGF by hypoxia, we have undertaken studies to determine the mechanism whereby hypoxia induces an increase in steady state levels of VEGF mRNA. A well-studied paradigm for hypoxic regulation of gene expression is erythropoietin, where studies have focused primarily on the transcriptional activation and have led to the identification of a 3′ *cis*-acting element that confers hypoxic regulation [12]. There are reported similarities between VEGF and erythropoietin regulation. For instance, both genes are up-regulated by exposure to cobalt chloride, a phenomenon attributed to the interaction of cobalt with a putative heme-binding oxygen sensor [13]. We initiated these studies, hypothesizing that the oxygen regulation of VEGF might parallel that of erythropoietin. However, we found virtually no increase in the transcriptional rate of VEGF following hypoxic exposure of human retinal pigment epithelial cells and instead observed a dramatic increase in VEGF mRNA stability following hypoxia.

2. Experimental

2.1. Cell lines and hypoxia protocol

Normal hRPE were isolated from donor eyes by enzymatic dissociation and used between passages 3–6 as described [4]. An immortalized hRPE cell line (tRPE), created by stable integration of a CMV-driven SV40 large T-antigen expression cassette, was established as described by others [14,15]. C6 astrocytoma and a mouse mammary epithelial cell line (C127I) were obtained from the American Tissue Culture Collection. hRPE, tRPE and C6 astrocytoma were cultured in Dulbeccos' modified Eagle's medium (DMEM) with 10% fetal calf serum (FCS), and penicillin and streptomycin. Mammary epithelial cells were cultured in DMEM with glucose supplement to 4.5 g/l. All cell lines were propagated in standard 10% CO_2, 21% O_2 incubators at 37°C. All tissue culture reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

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Abbreviations: VEGF, vascular endothelial growth factor; hRPE, human retinal pigment epithelial cells; tRPE, SV40 T antigen transformed human retinal pigment epithelial cells.

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An hypoxic environment was created by incubating the cells in a standard 10% CO_2 incubator infused with N_2 gas to create a constant 2% O_2 environment, as monitored by a Pro:ox oxygen regulator and sensor (Reming Bioinstruments, Redfield, NY). Normoxia is defined as 21% O_2 environment supplemented with 10% CO_2. All experiments were performed using confluent cell cultures.

2.2. Northern blot analysis

Total RNA was isolated from cultured cells by the modified acid-phenol method of Chomczynski and Sacchi [16], using RNAzol B (Tel-test). Fifteen micrograms of RNA/sample were fractionated by denaturing electrophoresis, and capillary blotted to charged nylon (Genescreen Plus, DuPont). Prehybridization and hybridization were carried out in 6× SSPE, 5× Denhardt's, 50% formamide, 1% SDS and 100 μg/ml sheared, denatured salmon sperm DNA. A 520 bp *NcoI*/*BglII* fragment of the human VEGF cDNA (a gift of Dr. Herbert Weich, Gesellschaft f. biotechnol. Forschung, Braunschweig, Germany), a 575 bp fragment encompassing the entire coding region of mouse VEGF-1 (provided by Georg Breier, Max Planck Institute, Bad Nauheim, Germany) [17]), a 400 bp fragment of large T-antigen, a 400 bp fragment encompassing the 3'UTR of the human β-actin cDNA [18] and a 280 bp fragment of bovine 28S ribosomal RNA were used to generate random-prime labeled probes for hybridizations [19]. After washing, blots were exposed to Kodak XAR-5 film with intensifying screens at −80°C. Quantification of autoradiographs was carried out with a phosphorimager using Image Quant software (Molecular Dynamics) and β-actin or 28S rRNA for normalization.

2.3. Nuclear run-on analysis of transcription

Transcriptional activation of the VEGF gene in confluent cultures of tRPE was examined by run-on analysis after normoxic, hypoxic, or phorbol ester (TPA; 100 nM) treatment of the cells for designated times. Cells and all reagents were kept on wet ice or at 4°C throughout the duration of the experiment. Cells (5×10^7/condition) were washed with ice-cold PBS and nuclei were directly scrape-isolated into NP-40 lysis buffer (10 mM Tris pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40 (v/v). Cells were pelleted at 300 × *g* and gently resuspended in 20 ml lysis buffer. Cell membranes were disrupted by 15 strokes of a prechilled Dounce homogenizer (type 'B' loose pestle). Nuclei were isolated by centrifugation (300 × *g*) and washed in 50 volumes of wash buffer (lysis buffer with 0.025% NP-40). After centrifugation, pelleted nuclei were resuspended in freeze buffer (20 mM Tris, pH 8.0, 75 mM NaCl, 0.5 mM EDTA, 1 mM DTT, and 50% glycerol), an aliquot examined by microscopy to confirm homogeneity and integrity of the preparation and the remaining samples were flash frozen in liquid N_2 at a concentration of $2–3 \times 10^8$ nuclei/ml, and stored at −80°C.

RNA synthesis in vitro was performed in the presence of approximately 2×10^7 nuclei in a reaction mixture of 50 mM Tris, pH 8.0, 5 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, 40 U Rnasin, 200 μM each of ATP, CTP and GTP, 200 μCi UTP (3000 Ci/mmol; New England Nuclear, Boston, MA) for 30 min at 26°C. Under these conditions, the rate of transcription is linear, as determined by parallel hybridizations with 5-fold dilutions of RNA probe (data not shown). Nascent labeled RNA was isolated by DNase digestion (25 U; 30 min at 37°C), proteinase K digestion (200 μg/ml) in 0.5% SDS for 60 min at 37°C, followed by hot phenol/chloroform extraction and repeated ethanol precipitation in 2.5 M ammonium acetate. Target DNA filters (Genescreen Plus, DuPont) were prepared by denaturation, neutralization and immobilization of 10 μg of linearized plasmid DNA harboring: a 520 bp fragment of human $VEGF_{165}$ (see above), a 400 bp fragment of the human β-actin 3' untranslated region (see above), a 1 kb fragment of the human basic FGF cDNA [4] and pBluescript II as a negative control.

Hybridizations were performed with 2×10^7 cpm/ml of labeled RNA in 5× SSPE, 50% deionized formamide, 2.5× Denhardt's, and 0.25% SDS for 40 h at 42°C. Filters were washed twice in 2× SSPE, 0.1% SDS for 1 h at 50°C, rinsed then incubated 1 h in 2× SSPE with 10 μg/ml RNase A, followed by two washes in 2× SSPE, 0.1% SDS for 1 h at 65°C. Filters were briefly dried and exposed to film with intensifying screens for 96 h at −80°C. Alternatively, filters were analyzed with a phosphorimager screen and results quantified using ImageQuant software. Results described are representative of 4 similar experiments in which time points ranging from 2 to 15 h of hypoxia treatment were examined.

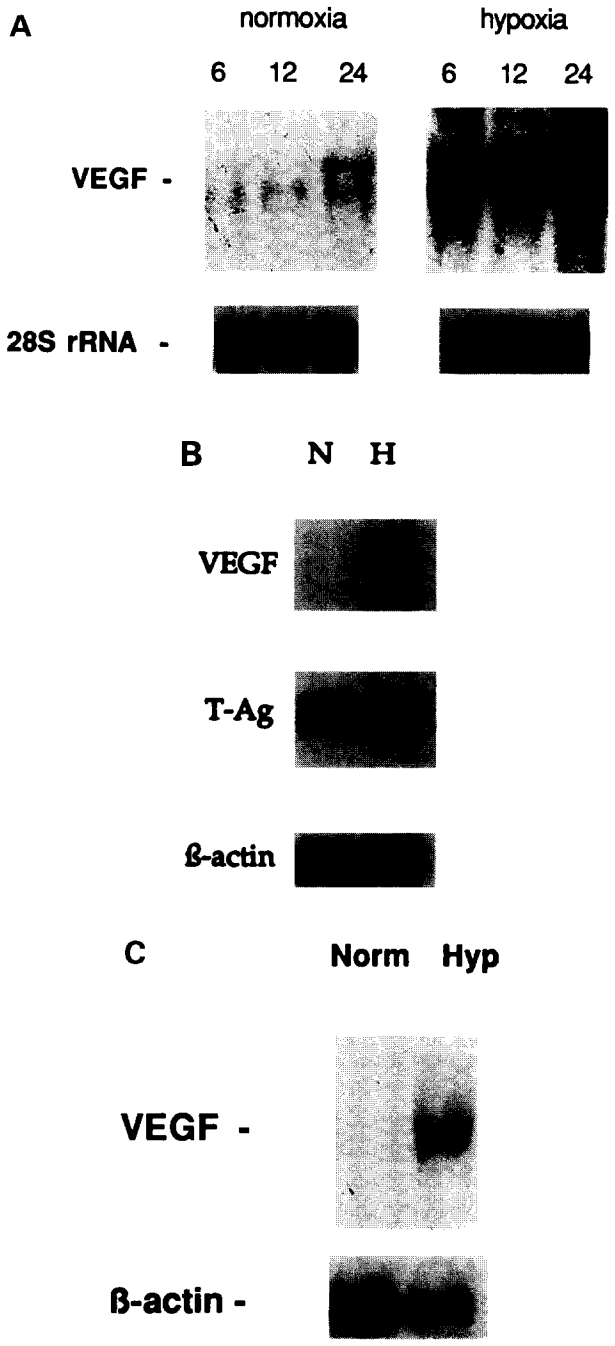

Fig. 1. Hypoxic induction of VEGF mRNA levels in epithelial cells. (A) hRPE were cultured for 6, 12 or 24 h in normoxia and hypoxia, and total RNA analyzed by Northern blot for VEGF mRNA. The same blot was stripped and hybridized with a 28S rRNA probe. (B) An immortalized hRPE cell line (tRPE) and a (C) mammary epithelial cell line were cultured for 15 h in normoxia or hypoxia, and total RNA analyzed for VEGF mRNA levels. Blots were stripped and hybridized with a T-antigen (B), and β-actin (B,C) cDNA probe.

2.4. Actinomycin D chase studies

Confluent cells were incubated in a normoxic or hypoxic environment, or in the presence of cobalt chloride (100 μM) for 15 h. The cells were then rinsed and refed with culture media containing actinomycin D (5 μg/ml) and incubated for an additional 1–8 h in standard culture conditions. Total RNA was

extracted and analyzed for VEGF and actin mRNA levels (see above). mRNA levels were quantified using a phosphorimager and ImageQuant software. These experiments were performed at least twice and representative results are shown.

3. Results

3.1. Effects of hypoxia on VEGF mRNA

VEGF mRNA levels in normal hRPE were elevated at least 10-fold after being cultured for 6 h in low oxygen and the steady state levels of VEGF mRNA continued to increase throughout the time course examined (up to 24 h) (Fig. 1A). As has been previously reported, two transcripts were observed (approx 3.8 and 4.0 kb) and most likely correspond to two of the four described alternatively spliced forms of VEGF mRNA. VEGF mRNA levels were similarly elevated in an immortalized human tRPE cell line (Fig. 1B) cultured under hypoxic conditions and in a mouse mammary epithelial cell line (data not shown). To date, all adherent cell types tested (more than 15 total) respond to hypoxia with an increase in VEGF mRNA. The exception is most endothelial cells, in which VEGF mRNA is undetectable. In contrast, β-actin mRNA (Fig. 1B; lanes 1 and 2), 28S rRNA (Fig. 1A; lanes 1–6) and T-antigen mRNA (Fig. 1B; lanes 1 and 2) levels in the various cell lines were not altered upon exposure to hypoxia.

The effects of hypoxia on VEGF gene expression are reproducibly detectable 3 h after the cells have been placed in an hypoxic environment; and there is continued accumulation of VEGF mRNA over the 15 h of a typical experiment (Fig. 2). The levels of VEGF mRNA induced by hypoxia, as well as that constitutively produced under normoxia, are dependent on protein synthesis since the addition of 10 μg/ml cycloheximide substantially decreased the levels of VEGF transcript levels in cells in normoxia or hypoxia for 15 h.

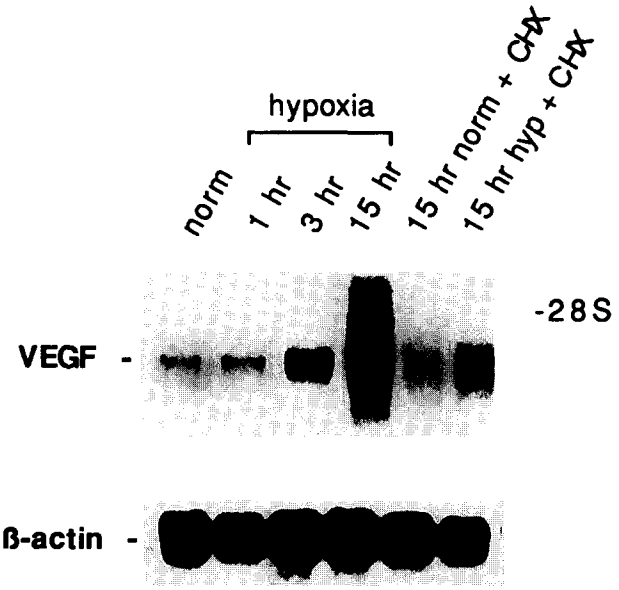

Fig. 2. Time course of hypoxic induction of VEGF mRNA levels and dependence on protein synthesis. tRPE were cultured for 0, 1, 3, and 15 h in hypoxia, or for 15 h in normoxia and hypoxia +/− the addition of 10 μg/ml cycloheximide. The same blot was analyzed for β-actin mRNA levels. The migration of 28S rRNA is marked.

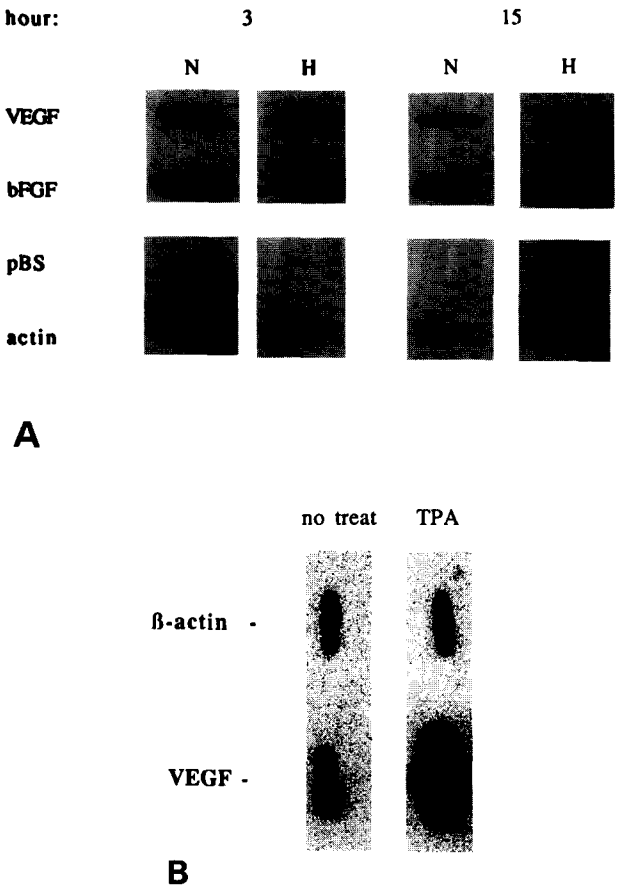

Fig. 3. Effect of hypoxia on VEGF transcriptional activity. (A) tRPE were incubated for 3 and 15 h in normoxic or hypoxic culture conditions, times when hypoxia-induced increases in VEGF mRNA are observed (see Fig. 2). Radiolabeled run-on RNA's were synthesized from isolated nuclei and hybridized to immobilized cDNA's for VEGF, bFGF, and actin. Background hybridization was assessed by hybridization of probes to a vector (pBSII) negative control. (B) For a positive control, tRPE exposed to phorbol ester (TPA) for 2 h were used in run-on analysis of VEGF and actin transcription.

3.2. Effect of hypoxia on the transcriptional rate of the VEGF gene

The current paradigm for hypoxic regulation of mammalian genes is provided by erythropoietin. Growth of the human hepatoma cell line HEP3B under hypoxic conditions leads to a 50-fold increase in steady state levels of erythropoietin mRNA [20]. Transcriptional activation of the erythropoietin gene has been a primary focus for understanding hypoxic regulation of erythropoietin gene expression, although an ill-defined role for mRNA stability has been suggested [21]. Therefore, to characterize of the cellular mechanisms regulating hypoxic induction of VEGF, tRPE cells grown for various time periods in normoxic or hypoxic conditions were used for nuclear run-on analysis. The apparent transcriptional rate for VEGF was compared to that for bFGF and the β-actin gene, which are not elevated in response to hypoxia [4]. Levels of baseline transcription assessed by hybridization of nascent, radiolabeled transcripts to immobilized cDNA's, varied slightly for the three genes studied but was significantly higher than background, non-specific probe hybridization to the vector control (Fig. 3A). In contrast to our expectations, levels of

VEGF transcripts in the run-on analysis showed little change during exposure of cells to hypoxia for 3 or 15 h, time points that correspond to periods in which VEGF mRNA levels are increasing (see Figs. 1A and 2). The most significant increase in the levels of VEGF transcripts ever detected in this assay (n=4) (approx 2.0 fold) was seen at 15 h of hypoxia, but this increase is also accompanied by similar increases in transcription for β-actin and bFGF. Thus, the slight increase seen in VEGF transcription at this late time point is not likely to be a specific response to hypoxia. Similar results were obtained when run-ons were conducted with cells grown in hypoxia for 2, 4, 6 and 12 h (data not shown). Phorbol ester treatment increases AP-1-mediated transcriptional activation of many genes, and has also been demonstrated to elevate VEGF mRNA levels [22,23]. To confirm that the assay utilized could detect elevation of VEGF transcription, 12 O-tetradecanoyl phorbol-13-acetate (TPA) treatment (2 h) was used as a positive control and was observed to lead to an approximately 4-fold increase in the VEGF transcriptional rate (Fig. 3B). Taken together, these data support a minimal role for transcriptional activation in the 10–15 fold induction of VEGF mRNA during hypoxia.

3.3. Effect of hypoxia on VEGF mRNA stability

The most likely alternative mechanism for regulation of VEGF mRNA by hypoxia is an increase in mRNA stability. Thus, actinomycin D chase studies were used to assess the rate of VEGF mRNA decay after exposure of tRPE to 15 h of normoxia or hypoxia. The efficacy of the actinomycin D treatment was assessed by quantifying incorporation of [^{3}H]UTP into total RNA. Results of these analyses indicated that more than 95% of RNA synthesis was inhibited over the time course of the study. The '0' point represents the level of VEGF mRNA following 15 h of culture in normoxia or hypoxia. As expected, mRNA from tRPE grown in low oxygen was significantly elevated (15-fold) compared to mRNA levels from cells grown under normoxic conditions (Fig. 4A). In the absence of transcription, VEGF mRNA from cells cultured in normoxia decays rapidly and is nearly undetectable by 2 h of the chase period. Examination of a more detailed time course revealed that VEGF mRNA was undetectable 1–2 h after cessation of transcription (data not shown). Unstable mRNA is a common characteristic of many growth-associated proteins such as onco-proteins and cytokines [24]. The relative instability of the VEGF transcripts is highlighted by comparison with the relatively stable mRNA of β-actin (Fig. 4A).

In contrast to the rapid decay of VEGF mRNA observed in cells cultured in normoxia, growth of cells in hypoxia led to a relative stabilization of VEGF mRNA, which remain constant until between 6–8 h, when a slight decrease (approx. 50%) was observed (Fig. 4). Overexposure of the blot examining VEGF mRNA stability in cells cultured under normal oxygen allowed detection of VEGF mRNA at 2 and 4 h following actinomycin D and was used for quantification and comparison of relative VEGF levels. A plot comparing relative mRNA levels as a function of time conveys the disparate rates of decay for VEGF mRNA under hypoxic versus normoxic culture conditions (Fig. 4C). The dramatic stabilization of VEGF mRNA in cells grown under hypoxic conditions represents a difference which could reasonably account for the elevated steady state VEGF levels during hypoxia.

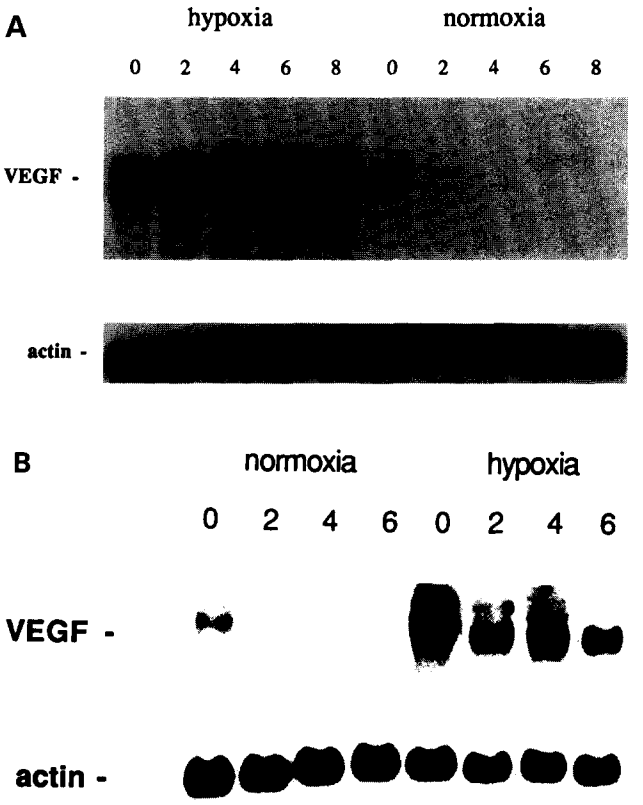

Fig. 4. (A) Effect of hypoxia on VEGF mRNA stability. tRPE were cultured in normoxic or hypoxic conditions for 15 h (time '0') and actinomycin D (5 μg/ml) was added to inhibit RNA synthesis. Cells were then incubated under normal culture conditions for 2, 4, 6 or 8 h, and VEGF mRNA levels were determined by Northern analysis. The same blot was stripped and hybridized with a control actin probe. The data presented is a 24 h exposure of the VEGF blot. (B) C6 astrocytoma were similarly analyzed for VEGF mRNA levels. (C) Decay rates for VEGF mRNA in tRPE and C6 astrocytoma cells cultured under normoxia (RPE, squares; C6 astrocytoma, circles) and hypoxia (RPE, diamonds; C6 astrocytoma, triangles). The RPE experiment was performed three times and the C6 experiment twice; representative data are shown.

To assess the universality of the hypoxia-induced increase in VEGF mRNA stability, VEGF mRNA decay rate was examined in a rat C6 astrocytoma cell line grown in hypoxia (Fig. 4B). Similar to the tRPE cell line, hypoxia led to an approximately 10-fold increase in steady-state VEGF mRNA levels (compare normoxia and hypoxia, time '0'). The VEGF mRNA was rapidly degraded in cells grown under normal oxygen conditions; levels were barely detectable after 2 h in the absence of transcription. As for the tRPE, hypoxia significantly stabilized VEGF mRNA levels (Fig. 4C).

Cobalt(II) is a metal which elevates VEGF mRNA to similar levels and with similar kinetics as hypoxia [25,26]. As it has been proposed that cobalt and hypoxia stimulate gene expression through the same mechanisms [25], we examined VEGF mRNA decay in the presence of cobalt. As for hypoxia, 15 h of cobalt chloride (100 μM) exposure led to a 10-fold increase in steady-state (time '0') VEGF mRNA levels but had a minimal effect on the stability of VEGF mRNA (Fig. 5A and B). These results suggest that, unlike hypoxia, the increases in VEGF mRNA associated with cobalt cannot be accounted for by mRNA stability alone.

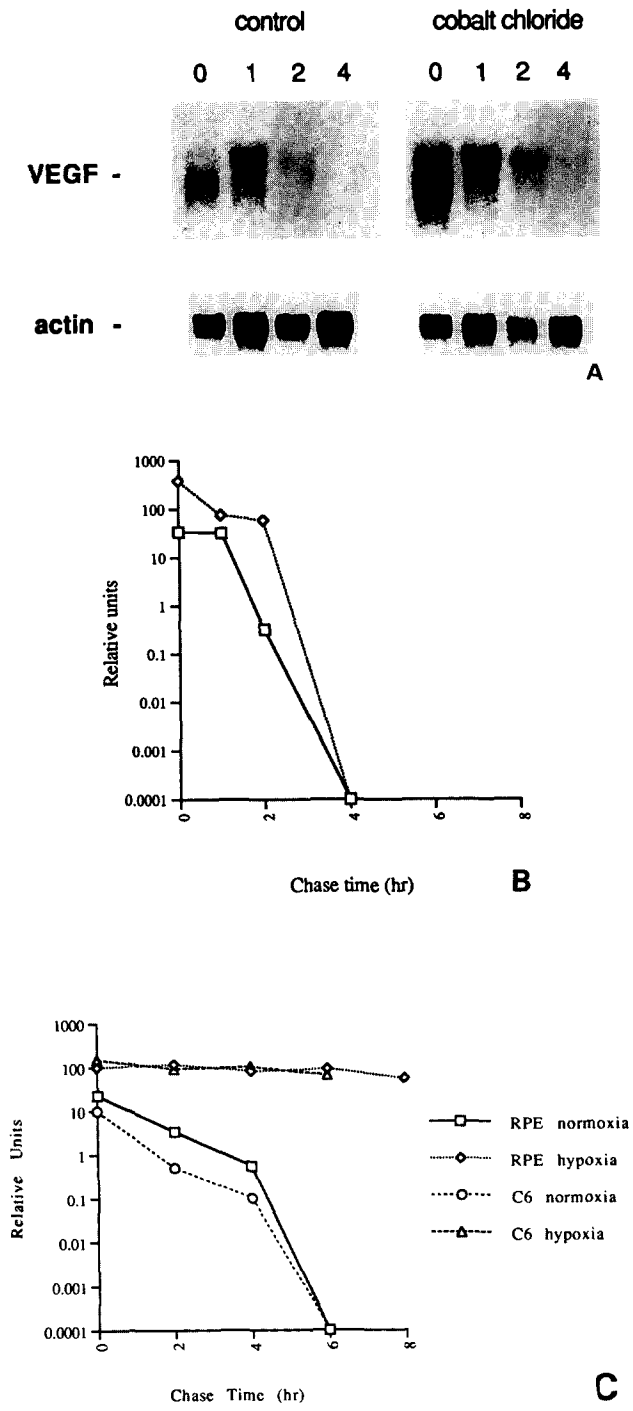

Fig. 5. Effect of cobalt chloride treatment on VEGF mRNA stability. (A) tRPE were cultured in control media, or media with 100 μM cobalt chloride for 15 h. Cells were refed with control media supplemented with 5 μg/ml actinomycin D, incubated for 0, 1, 2, or 4 h, and total RNA was isolated and analyzed for VEGF mRNA levels. The same blot was stripped and hybridized with a control actin cDNA probe. (B) Graphic representation of the decay rates for VEGF mRNA in control and cobalt treated cultures.

4. Discussion

We and others have shown that the expression of VEGF is induced by low oxygen or hypoxia [3,4]. Using primary cultures of hRPE, we have observed a 10- to 15-fold increase in VEGF mRNA in cells grown in a low oxygen environment. Similarly, we have rendered retinal tissue ischemic (and therefore, hypoxic) and observed an increase in ocular angiogenesis that was spatially and temporally correlated with an elevation in VEGF levels [9]. We were, therefore, interested in the mechanism whereby hypoxia might lead to the dramatic increases in steady state levels of VEGF mRNA. As a model for the possible mechanism, we looked to studies that had been conducted with erythropoietin, the hormone which regulates erythrocyte production. Similarities between the regulation of VEGF and erythropoietin gene expression were suggested by the fact that the expression of both genes is induced by cobalt chloride. Cobalt is suspected to act by forcing an oxygen-sensing hemeprotein into the deoxy conformation, thereby mimicking hypoxia [13]. Studies to date examining the molecular mechanisms controlling erythropoietin levels have focused primarily on transcriptional regulation [12,27,28].

However, run-on analysis of normal and transformed RPE grown in low oxygen revealed minimal transcriptional activation of the VEGF gene throughout an extensive time course (2–15 h). Our confidence in this negative result is strengthened by the observation of a significant increase in VEGF transcription by phorbol ester, which has been shown to increase VEGF mRNA levels. Since transcriptional activation does not appear to account for the hypoxia-induced increase in VEGF mRNA, we examined the possibility that elevated mRNA levels were the result of enhanced mRNA stability. Indeed, analysis of VEGF mRNA decay in cells cultured under normoxic and hypoxic conditions revealed dramatic differences. When the tRPE cells were cultured in normoxia, VEGF transcripts, like the mRNA of other cytokines [29], had a relatively short half-life, approximately 30–60 min. In contrast, the half-life of VEGF in tRPE grown in hypoxia was extended to 8 h. Thus, the increase in steady state levels of VEGF mRNA in RPE following hypoxia appears to be due, in large part, to a stabilization of VEGF transcripts in the face of relatively constant transcriptional rate.

In a recent report, Minchenko and coworkers [26] suggest a role for transcriptional activation in the hypoxia-induced increase in VEGF mRNA. Using transient transfections assays in HeLa cells, *cis*-elements, which appear to possess hypoxia- and cobalt-responsiveness, were identified in both the 3′ and 5′ flanking regions of the human VEGF gene. The 3′ enhancer shares limited homology with a sequence previously identified in the erythropoietin 3′ flanking region. However, because there was no demonstration of transcriptional activation (i.e. run-on analysis) in this report, the relative contribution of transcription in this system is difficult to assess. Without information on the activity of the proposed trans-activating complexes in the context of normal chromatin architecture, results obtained by splicing gene fragments to reporter genes must be interpreted with caution.

Our data suggest an extremely modest role for transcriptional activation of the VEGF gene in tRPE, but instead indicate that hypoxia-induction of VEGF mRNA occurs predominantly via changes in mRNA stability. It is possible that VEGF regulation differs among cell lines or species and that transcriptional activation may contribute to the hypoxic induction of VEGF mRNA in different experimental systems. However, our observation in rat C6 astrocytoma (the cells used in early studies demonstrating hypoxic induction of VEGF [3]) of hypoxia-induced stabilization of VEGF mRNA comparable to that ob-

served in the RPE cells, supports a general role for hypoxia-induced mRNA stability.

The observation that cobalt chloride treatment leads to elevated VEGF mRNA has led others to conclude that cobalt and hypoxia act via the same mechanisms to increase the steady state levels of VEGF mRNA [25]. Our finding that the cobalt-induced increase in steady state levels of VEGF mRNA is not associated with significant VEGF mRNA stabilization distinguishes the effects of cobalt from those of hypoxia. Cobalt (II) has pleiotropic effects on tissues and cultured cells, including generation of reactive oxygen species that lead to DNA damage [30,31], lipid peroxidation [32] and metabolic dysfunction [33]. Taken together, these data suggest that cobalt and hypoxia exert their effects on VEGF through separate, though potentially overlapping pathways.

mRNA stability has been well-documented to play a role in the regulation of transferrin receptor levels [34,35] and provides the best-studied paradigm of inducible mRNA stability to date. Transferrin receptor synthesis is depressed when iron is plentiful and is elevated when iron levels are reduced. The effects of iron on transferrin mRNA have been shown to be mediated primarily via a sequence in the 3′ UTR of the mRNA [36]. Sequence of the VEGF 3′UTR is not yet available so it is not possible to make predictions regarding secondary structure(s) which might influence message stability. Although sequences in the 5′ flanking region of transferrnin have been shown to mediate a 2- to 3-fold increase in transferrin receptor transcription in iron-deficient cells, deletion of the 3′ UTR sequence eliminates the majority of iron regulation even if the promoter is left intact. Thus, VEGF and the transferrin receptor may be similar in this regard; although there appear to be elements which are able to confer hypoxia-induced transcriptional control, regulation, at least under the conditions of our experiments, is predominantly at the level of mRNA stability.

Regulation of VEGF mRNA at the level of stability seems logical in light of the proposed function of this factor as an initiator of angiogenesis. Blood vessels, ordinarily quiescent tissues, undergo swift, controlled bursts of growth during such physiological processes as corpus luteal maturation and wound healing. High turnover of growth related mRNA's, such as VEGF, provides an exquisitely responsive system [37]. We postulate that hypoxia-induced increases in mRNA stability permit the sustained increases in VEGF mRNA levels required to support a neovascular response.

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