ABSTRACT

Vascular endothelial growth factor (VEGF) and its tyrosine kinase receptors VEGFR-1 (flt-1) and VEGFR-2 (flk-1/KDR) are key mediators of physiological and pathological angiogenesis. They are expressed in most tissues during embryonic development but are down-regulated in the adult, when angiogenesis ceases. Up-regulation of VEGFR-2 and of VEGF are observed in many pathological conditions under which angiogenesis is reinduced. A major regulator of VEGF expression is hypoxia. Although the temporal expression pattern of VEGF-2 parallels VEGF expression to a high extent, little is known about its regulation. Here, we show that VEGFR-2 is highly expressed in early postnatal mouse brain but is down-regulated commencing at postnatal day 15 (P15) of mouse brain development and is hardly detectable in P30 mouse brain. Using P30 mouse brain slices, we observed that hypoxia up-regulates VEGFR-2 in the slices but not in human umbilical vein endothelial cells, suggesting the presence of a hypoxia-inducible factor in the murine neuroectoderm that up-regulates VEGFR-2. To identify the factors involved, normoxic P30 cerebral slices were cultured with growth factors that are either hypoxia-inducible (e.g., PDGF-BB, erythropoietin, and VEGF) and/or are known to act on endothelial cells (e.g., PDGF-BB, VEGF, and PIGF). Exogenously added recombinant VEGF led to an up-regulation of VEGFR-2 expression, which could be inhibited by preincubation with a neutralizing anti-VEGF antibody. Addition of PDGF-BB, PIGF, and erythropoietin had no effect on VEGFR-2 expression. Our results suggest a differential but synergistic regulation by hypoxia of VEGF and VEGFR-2: a direct induction of VEGF that subsequently up-regulates VEGFR-2 in endothelial cells. This autoenhancing system may represent an important mechanism of tumor angiogenesis.

INTRODUCTION

VEGF and its signal transducing tyrosine kinase receptor VEGFR-2 (flt-1/KDR) are major mediators of developmental, hypoxia/ischemia-induced and tumor-induced angiogenesis (for reviews see Refs. 1–4). The importance of VEGFR-2 and its ligand VEGF for the development of the vascular system has been shown in transgenic mice that carry targeted disruptions of the respective genes. VEGFR-2-deficient mice fail to develop a vascular system and die between E8.5 and E9.5, at the time at which the blood islands, which harbor the precursors for endothelial cells and blood cells (hemangioblasts), are formed (5). Mice deficient in VEGF die already in the heterozygous state, suggesting that normal embryonic development is dependent on VEGF dosage (6, 7). A role of VEGF and VEGFR-2 in pathological angiogenesis (i.e., the growth of new blood vessels observed in diseases such as proliferative retinopathies, psoriasis, rheumatoid arthritis, myocardial infarction, peripheral arterial stenosis, and tumor growth) has been suggested by studies showing up-regulation of VEGF in a variety of cell types, such as neural cells (8), epithelial cells (9, 10), monocytes/macrophages (11), myocytes (12), and tumor cells (13, 14) and of VEGFR-2 in endothelial cells of the respective tissues (15, 16). A functional role for VEGF in angiogenesis in vivo has been demonstrated by i.v. injection of recombinant VEGF in a rabbit model of peripheral arterial disease, in which collateral formation was induced (17). On the other hand, depletion of VEGF by neutralizing antibodies or antisense strategies significantly inhibits tumor growth in animal models (18–20). In rats and mice, we have shown that VEGFR-2 is necessary for angiogenesis in a variety of tumors because introduction of a signaling-deficient VEGFR-2 in endothelial cells led to inhibition of tumor angiogenesis and tumor growth in vivo (21, 22).

It has been shown that VEGF is hypoxia-inducible in a wide variety of cell types (12). Hypoxic up-regulation of VEGF is achieved by a dual mechanism that includes transcriptional activation and an increase in mRNA stability (23). A binding site for hypoxia-inducible factor 1 has been identified in the 5′ untranslated region of the murine and human VEGF gene which accounts for transcriptional activation (24). The site responsible for increased mRNA stability is not well defined but is probably located on the 3′ untranslated region of the VEGF gene (25). Although the hypoxia inducibility of VEGF in vitro is clearly established, little is known about the regulation of VEGFR-2. VEGFR-2 is one of two receptor tyrosine kinases (the other is designated flt-1 or VEGFR-1) that bind VEGF with high affinity (26). Expression of VEGFR-1 and VEGFR-2 appears to be confined to the endothelial cell lineage in vivo (26, 27). VEGFR-2 is expressed in endothelial cells during embryonic development but is down-regulated in most adult tissues, when angiogenesis ceases, thus suggesting a role in developmental angiogenesis (26). In vitro, binding of VEGF to VEGFR-2 leads to receptor phosphorylation and a mitogenic response (26, 28). Expression of VEGFR-2 parallels expression of VEGF to a remarkably high extent. Both genes are expressed in developing tissues, are down-regulated, with few exceptions, in adult tissues and are up-regulated under pathological conditions, albeit in different cell types. For example, in transplanted intracerebral rat gliomas, VEGF is expressed in the tumor cells to a much higher extent than in the normal surrounding brain (29). Similarly, VEGFR-2 is expressed only in endothelial cells within or in the immediate surrounding of the tumor, but not in endothelial cells in the normal surrounding brain (29). A common mechanism of VEGF and VEGFR-2 regulation seems therefore likely. In vitro studies of VEGFR-2 regulation are hampered by the fact that most cultured endothelial cells express high amounts of VEGFRs and thus do not necessarily reflect the in vivo situation. We therefore developed a mouse cerebral slice culture system that enabled us to study endothelial cells within their organotypic cellular environment. Using this system, we investigated postnatal VEGFR-2 expression and studied factors that up-regulate VEGFR-2. We show here that hypoxic murine neuroectoderm is capable of up-regulating VEGFR-2 in endothelial cells in P30 mouse brain slices and that this effect is due to a paracrine induction by its ligand VEGF.

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2 To whom requests for reprints should be addressed. Phone: 49-761-270-5107; Fax: 49-761-270-5050; E-mail: plate@rz1.ukl.uni-freiburg.de.
3 The abbreviations used are: VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; PECAM, platelet-endothelial cell adhesion molecule; PBSS, PBS with 20% normal goat serum and 0.1% Triton X-100; PBT, 0.2% BSA and 0.1% Triton X-100 dissolved in PBS; HUVEC, human umbilical vein endothelial cell; PDGF, platelet-derived growth factor; PIGF, placenta growth factor; P, postnatal day; E, embryonic day; EGM, endothelial cell growth medium.

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MATERIALS AND METHODS

Cerebral Slice Cultures. Male and female BALB/c mice (Charles River, Sulzfeld, Germany) in postnatal stages P0–P30 were decapitated and the brain removed under sterile conditions. After dissecting away the brain stem and cerebellum, the brain was cut into 400-μm-thick slices by means of a tissue chopper. The sliced brains were immediately put into ice-cold PBS (Biochrom, Berlin, Germany) and the slices were carefully dissociated with sterile instruments and transferred into 24-well plates (Costar, Germany). For the studies on postnatal PECAM and VEGFR-2 expression, slices were fixed immediately and further processed for whole mount immunohistochemistry (see below).

For the studies of VEGFR-2 up-regulation, slices from P28–P32 mice were cultured in EGM supplemented with 2% FCS, 0.1 ng/ml recombinant human epidermal growth factor, 1.0 ng/ml recombinant human basic fibroblast growth factor, 1.0 μg/ml hydrocortisone, 50 μg/ml gentamicin, 2.5 μg/ml amphoter- icine B, and 0.4% endothelial cell growth supplement/heparin (Promo Cell, Heidelberg, Germany) under normoxic or hypoxic conditions (see below) or EGM was supplemented with various growth factors and their respective antibodies (see Table I). All experiments were carried out with culture times of 6 and 24 h and repeated at least three times. Slices were then processed for whole mount immunohistochemistry, as described below.

Whole Mount Immunohistochemistry of Cerebral Slice Cultures. On day 1, slices were washed twice in PBS at room temperature and fixed overnight in 4% paraformaldehyde (Merck, Darmstadt, Germany) at 4°C. Slices were washed twice in PBS and dehydrated through 25, 50, 75, 100, and 100% methanol (15 min each), at room temperature. Slices were then blocked for 4 h in 5% H2O2 dissolved in 100% methanol at room temperature, washed in 100% methanol and stored overnight or for a maximum of 2 weeks in 100% methanol at −20°C. On day 2, slices were rehydrated through 100, 75, 50, and 25% methanol and PBS (twice), 15 min each. Slices were then incubated in PBSST for 15 min at room temperature and subsequently stored in PBSST at 4°C overnight. On day 3, slices were incubated overnight at 4°C with the following antibodies: a hybridoma supernatant containing monoclonal rat antiserum PECAM antibody (dilution 1:2000 in PBSST; a gift from Dr. E. Dejana, Institute Mario Negri, Italy) or a monoclonal rat antiserum to VEGFR-2 antibody (diluted 1:1000 in PBSST; a gift from Dr. Hiroshi Kataoka, Department of Molecular Genetics, Kyoto University, Japan). For control purposes, slices were incubated in PBSST alone. On day 4, slices were washed in PBSST at room temperature (six times) and subsequently incubated overnight at 4°C with a goat antirat biotinylated secondary antibody (diluted 1:2000 in PBSST; final concentration, 0.5 μg/ml; purchased from Dianova, Hamburg, Germany). On day 5, slices were washed in PBSST at room temperature (six times) and subsequently incubated overnight at 4°C with Vectastain ABC Elite Kit according to the manufacturer’s instructions.

Table 1 Effect of growth factors and antibodies on VEGFR-2 and PECAM expression in P30 mouse brain slices

<table>
<thead>
<tr>
<th>Factors added</th>
<th>VEGFR-2</th>
<th>PECAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control EGM)</td>
<td>− ‡</td>
<td>+</td>
</tr>
<tr>
<td>Erythropoietin, 10, 100 or 1000 units/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Erythropoietin, 1000 units/ml + anti-erythropoietin Ab, 20 or 200 μg/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-erythropoietin Ab, 20 or 200 μg/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF, 1 or 10 ng/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-AA, 1, 10, or 100 ng/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-AA, 1, 10, or 100 ng/ml, + PDGF-A antiserum, 20 μl/ml</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-BB, 1, 10, or 100 ng/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-BB, 1, 10, or 100 ng/ml, + PDGF-B antiserum, 20 μl/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-A antiserum, 20 μl/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PDGF-B antiserum, 20 μl/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VEGF165 or 165 1, 10, or 100 ng/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>VEGF165, 1, 10, or 100 ng/ml + anti-VEGF antibody, 5 or 10 μg/ml</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-VEGF antibody, 5 or 10 μg/ml</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

‡ + and −, up-regulation and no up-regulation, respectively, of VEGFR-2 compared to P30 control slices.

On day 6, slices were washed three times at room temperature in PBS (10 min each) and subsequently three times (10 min each) at room temperature in PBT. Slices were then incubated for 100 μl per well of a solution containing 0.28 mg of dianamobenzidine (Sigma, Munich, Germany) dissolved in 10 ml of PBT, 100 μl of NCI (500 mg/ml stock solution; Sigma), and 10 ml of H2O2 (30%). After 2–4 min, the reaction was stopped with PBT, and slices were washed in PBT (two times for 5 min each) at room temperature, then washed in PBS (two times for 5 min each) at room temperature and subsequently mounted in Kaisers glycerin/gelatin (Merck) solution. Slices were immediately photographed as a dark field image using a Zeiss Axioskop (Oberkochen, Germany) and a Kodak Ektachrom film.

In situ Hybridization. We compared VEGF-2 expression observed in cerebral slices with in situ hybridization analyses from fresh frozen sections in the most relevant postnatal stages. Male or female BALB/c mice from postnatal stages P10, P15, P20, and P30 were decapitated, and the brain was removed. The brain was embedded in Tissue Tek (Miles) and immediately snap frozen in liquid nitrogen and stored at −70°C until further use. Cryostat sections (10 μm thick) were used for in situ hybridization with a 35S-labeled fik-1 cRNA probe as described previously (26, 27). Slides were exposed for 10 days at 4°C, developed, and counterstained with toluidine blue.

Cell Culture, RNA Extraction, and Northern Analysis. HUVECs were freshly isolated and cultured in EGM as described previously (30) in 2% gelatin-coated plates.

Medium was changed 24 h prior to the experiments. HUVECs were cultured in 100-mm Petri dishes until they achieved subconfluency. Cells were then cultured for 24 h at 37°C in a incubator with 21% oxygen and 5% CO2 (normoxia) or in an anaerobic gas chamber (see below). Total RNA was extracted from cells with the Qiagen RNA isolation kit (RNeasy Total RNA kit, Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Aliquots of RNA (10 μg) were electrophoresed in a 1.5% agarose gel containing 15% formaldehyde and subsequently transferred to a Duralon membrane (Stratagene, La Jolla, CA) in 20× SSC. Filters were cross-linked with UV light (0.4 J/cm2) and hybridized at 68°C using hybridization solution (QuickHyb, Stratagene) and a random-primed 32P-labeled DNA using a 1.4-kbp fragment encoding part of the extracellular domain of the human VEGFR-2 gene (32). For control of RNA loading we used a cDNA fragment coding for chicken β-actin.

Hypoxia Assay. For the hypoxia experiments, HUVECs and cerebral slices were cultured in a hypoxic (approximately 0.2% O2) gas chamber (Becton Dickinson) as described previously (29). For control purposes, cells and slices were cultured for the same time periods under normoxic conditions (21% oxygen, 5% CO2). Incubation time was 24 h for HUVECs and 6 and 24 h for cerebral slices.

Incubation of Cerebral Slices with Cytokines and Antibodies. P28–P32 cerebral slices were freshly prepared as described above and cultured for 1 h in EGM. After 1 h, medium was removed, and fresh EGM, which was supplemented with the following growth factors and/or antibodies, was added: b-actin-derived recombinant murine VEGF164 or recombinant human VEGF165 (both gifts of Dr. H. Weich, German Biotechnology Research Center, Braunschweig, Germany); either with or without neutralizing monoclonal anti-VEGF antibody (AB-293-NA, R&D Systems, Inc., Minneapolis, MN); recombinant PDGF-AA and PDGF-BB, either with or without their respective neutralizing antisera (a gift of Dr. C. Heldin, Biomedical Center, Uppsala, Sweden); recombinant erythropoietin (Cilag, Sulzbach, Germany); either with or without neutralizing monoclonal antierythropoietin antibody (AB-286-NA, R&D); and recombinant murine PI GF (a gift of Dr. H. Weich, Braunschweig, Germany). The concentrations of cytokines and antibodies used and the various cytokine/antibody combinations tested are shown in Table I.

RESULTS

Expression of VEGFR-2 in P0–P30 Mouse Brain Cerebral Slices. Because angiogenesis is a complex process that involves interaction of several cell types, we developed an organotypic tissue culture system to study murine brain angiogenesis. Brain slices from P0, P5, P10, P15, P20, P25, and P30 mice stained for VEGFR-2 showed a strong labeling of vessels in mice from P0 up to P15 and a gradual decline thereafter. VEGFR-2 expression was measured by in situ hybridization using brain sections of P0–P30 control slices.
<table>
<thead>
<tr>
<th>VEGFR-2</th>
<th>PECAM</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td>P0</td>
<td>P0</td>
<td>P0</td>
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<td>P5</td>
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LIGAND-INDUCED UP-REGULATION OF VEGFR-2

Research.
LIGAND-INDUCED UP-REGULATION OF VEGFR-2

Fig. 1. A, expression of VEGFR-2 and PECAM in freshly fixed mouse brain slices from P0 to P30. VEGFR-2 and PECAM expression was confined to endothelial cells. VEGFR-2 expression was high up to P15 and gradually declined thereafter. In P30 brain, VEGFR-2 was hardly detectable. In contrast, PECAM was constitutively expressed in all postnatal stages examined. Bar, 20 μm. B, expression of VEGFR-2 mRNA on frozen sections of mouse brain from P10, P15, P20, and P30. Similar to the results obtained by whole mount immunohistochemistry on cerebral slices, VEGFR-2 was gradually down-regulated after P10 and was hardly detectable at P30.

hardly detectable in P 30 mice (Fig. 1A). Our immunohistochemical data were confirmed by an in situ hybridization study on frozen sections of postnatal stages P10, P15, P20, and P30 that showed a similar temporal and spatial expression pattern of VEGFR-2 mRNA compared to the protein (Fig. 1B). In contrast, PECAM was constitutively expressed in the vasculature of all postnatal stages examined (Fig. 1A) and was used as a control in subsequent studies.

**Hypoxia Inducibility of VEGFR-2 in Cultured Cerebral Slices.** Because most if not all endothelial cells in culture express high levels of VEGFR-2, we compared cerebral slice cultures from P30 mice and cultured HUVECs for VEGFR-2 expression. We first tested whether hypoxia up-regulates VEGFR-2. In three independent experiments, we observed that HUVECs cultured under hypoxic conditions down-regulate VEGFR-2 mRNA, whereas VEGF mRNA was up-regulated (Fig. 2). We then cultured cerebral slices from P 30 mice under normoxic and hypoxic conditions. Blood vessels in hypoxic cerebral slices showed a significant increase in VEGFR-2 but not in PECAM expression compared to their normoxic controls (Fig. 3). These findings are consistent with the hypothesis that VEGFR-2 regulation occurs via a paracrine mechanism as we previously proposed in a rat glioma model (29) and support the observation of Brogi et al. (31) that the factor responsible for VEGFR-2 up-regulation is hypoxia inducible.
**INDUCTION OF VEGFR-2 BY VEGF.** To identify the factor responsible for VEGFR-2 up-regulation in murine neuroectoderm, we tested several candidate factors. PDGF-A and PDGF-B are expressed in neuroectoderm (33). PDGF-B is hypoxia inducible (34) and acts on endothelial cells (35), which can express PDGF-β (36, 37). PIGF is an endothelial cell-specific growth factor that binds to VEGFR-1 but not VEGFR-2 (38). PIGF may act synergistically with VEGF on endothelial cells (38). PIGF is reported to be down-regulated by hypoxia in vitro (39), but in vivo data are not available. Erythropoietin is a hematopoietic growth factor that has been shown to be hypoxia inducible in murine, monkey, and human brain (40). Erythropoietin receptors have been demonstrated in endothelial cells (41). VEGF is expressed in neuroectoderm (42) and is hypoxia inducible (12), and VEGFRs are present on brain endothelial cells (16, 26, 27, 43). We therefore investigated the effects of recombinant PDGF-AA and PDGF-BB, PIGF, erythropoietin, and VEGF on VEGFR-2 expression in normoxic cerebral slices of P30 mice.

An induction of VEGFR-2 was observed by recombinant VEGF164 (murine) or VEGF165 (human), whereas PDGF-AA, PDGF-BB, and PIGF had no activity (Table 1; Fig. 4). VEGF-2 up-regulation induced by recombinant VEGF appeared to be stronger than by hypoxia. A possible explanation could be a higher concentration of exogenously added VEGF compared to the endogenous level achieved by hypoxic up-regulation. Expression of PECAM was unaltered in hypoxic slices and in slices treated with growth factors and/or antibodies compared to P30 control slices (Table 1).

**INHIBITION OF VEGF-INDUCED VEGFR-2 UP-REGULATION BY A NEUTRALIZING ANTI-VEGF ANTIBODY.** Preincubation of VEGF165 with a neutralizing monoclonal anti-human VEGF antibody abolished the VEGFR-2 induction. Incubation with the antibody alone had no effect on VEGFR-2 or PECAM expression. In contrast, preincubation of PDGF-AA, PDGF-BB, and erythropoietin with specific neutralizing antibodies did not alter VEGFR-2 expression when compared to the peptides alone (Fig. 4; Table 1). Attempts to inhibit the endogenous VEGF activity by neutralizing antibodies in hypoxic brain slices showed inconsistent results, probably due to inefficient penetration of the antibody.

**DISCUSSION**

In the mouse, VEGF-2 expression is detected for the first time around E7.5 in blood islands (44). At a later point during embryonic mouse development, most, if not all, endothelial cells express VEGF-2 (26). This is particularly apparent in the nervous system, in which endothelial cells that invade the neuroectoderm show strong VEGF-2 expression (26). VEGF-2 expression starts to decline around P15, a period during which endothelial cell proliferation is rapidly down-regulated in the mouse brain (45). Angiogenesis is turned on in the adult organism under a variety of pathological conditions (46). In most, if not all, pathological conditions in which angiogenesis is ongoing, VEGF-2 is up-regulated in endothelial cells, whereas the ligand VEGF is up-regulated in nearby non-endothelial cells, consistent with a paracrine mechanism.

The aim of our study was to identify factors that up-regulate VEGFR-2 in endothelial cells. Because isolated cultured endothelial cells, such as HUVECs, express high amounts of VEGFRs in vitro, we used an organotypic cerebral slice culture system, which leaves endothelial cells in close contact with their cellular environment. In this system, freshly isolated cerebral slices from P20–P30 showed no detectable expression of VEGF-2. We show here that hypoxic neuroectoderm and recombinant VEGF but not PIGF, erythropoietin, PDGF-AA, and PDGF-BB up-regulate VEGFR-2. This observation is not unexpected. First, VEGF-2 and VEGF show a remarkably high overlap in their temporal expression pattern during embryonic development, in the adult organism and in pathological conditions, which suggests an autoregulatory mechanism. Second, our observations and those by Brogi et al. (31) suggest that VEGF-2 is up-regulated by a secreted, hypoxia-inducible factor produced by nonendothelial cells. Third, the factor that induces VEGF-2 should also operate under nonhypoxic conditions. This hypothesis is based on the observation that in hemangioblastomas, human brain tumors that develop as a consequence of loss of function of the von Hippel-Lindau tumor suppressor gene (47), a high expression of VEGF-2 is observed (48), although no signs of hypoxia are present (for review see Ref. 49). Loss of function of the von Hippel-Lindau gene has recently been shown to induce the up-regulation of several hypoxia-regulated genes, including VEGF (50, 51), under normoxic conditions. Finally, studies of VEGF-2 up-regulation induced by retroviruses encoding avian VEGF122 in the developing chick limb bud (52) and by recombinant human VEGF123 in the chorioallantoic membrane (53) suggested that VEGF may be involved in the regulation of its own receptor. It is unknown whether VEGF-1 is required for up-regulation of VEGFR-2. Our finding that PIGF, which selectively binds to VEGFR-1, does not induce VEGFR-2, is, however, not in favor of this notion.

It remains unclear whether hypoxia itself up-regulates VEGFR-2 in HUVECs. Although VEGF shows hypoxic up-regulation, the amount of VEGF produced by hypoxic HUVECs may not be sufficient to induce the receptor in this culture system. Another possibility, suggested by Waltenberger et al. (54), is an entirely posttranscriptional up-regulation of VEGF-2 by hypoxic mechanisms. However, Brogi

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**Fig. 2.** Down-regulation of VEGFR-2 mRNA by hypoxia in HUVECs in vitro; Northern analysis of 10 μg of total RNA derived from normoxic and hypoxic HUVECs.
et al. (31) were unable to show VEGFR-2 up-regulation in HUVECs by exogenously added VEGF. Apparently, the mechanisms for VEGFR-2 regulation are altered in cultured endothelial cells. As we show, cerebral slice cultures may be useful to overcome this problem.

Our results suggest a tight control of angiogenesis mediated by VEGF, which up-regulates its own receptor. Vascularization in a given tissue may therefore depend on the endogenous level of VEGF, which is regulated by the intracellular oxygen tension and by cytokines. Autoenhancement of receptor-ligand systems has been described for a variety of receptors, including the follicle-stimulating hormone receptor (55), the dopamine D1 receptor (56), the transferrin receptor (57), and the activin receptors I and IIB (58). However, the exact nature of the ligand-induced receptor up-regulation is not known. Our findings shed new light on the mechanisms that regulate
Fig. 4. Effect of various growth factors and antibodies on VEGFR-2 expression in P30 mouse brain slices. Of the growth factors tested, only VEGF was able to induce VEGFR-2 expression. PECAM expression was assessed in parallel in all experiments and did not show any alteration compared to controls. All pictures were taken at the same magnification; bar, 20 μm.

developmental and pathological angiogenesis and may be important in developing therapeutic strategies to inhibit pathological angiogenesis.

ACKNOWLEDGMENTS

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Up-Regulation of flk-1/Vascular Endothelial Growth Factor Receptor 2 by Its Ligand in a Cerebral Slice Culture System

Christine Kremer, Georg Breier, Werner Risau, et al.


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