Neuroendocrinology Letters ISSN 0172–780X

ORIGINAL ARTICLE

Focal ischemia in the cerebral cortex has an effect on the neurohypophysis

II. Angiogenesis in the neurohypophysis is a consequence of the focal ischemia in the cerebral cortex

Malgorzata Frontczak-Baniewicz & Barbara Gajkowska

Laboratory of the Cell Ultrastructure, Medical Research Centre Polish Academy of Sciences, Pawinskiego St. 5, 02-106 Warsaw, Poland.

Correspondence to: Malgorzata Frontczak-Baniewicz, Ph.D.
Laboratory of the Cell Ultrastructure, Medical Research Centre Polish Academy of Sciences, Pawińskiego St. 5, 02-106 Warsaw, Poland.
TEL.: +48 22 668-5277; +48 22 608-6412
FAX: +48 22 668-55-32
E-MAIL: gosia@cmdik.pan.pl

Submitted: *February 14, 2001*
Accepted: *March 16, 2001*

Key words: **capillary vessel; basement membrane; extracellular matrix; angiogenesis**

*Neuroendocrinology Letters 2001; **22**:87–92 pii: NEL220201A02*

Abstract

OBJECTIVES: Focal ischemia in the cerebral cortex has an effect on neurohypophysis. The morphological changes of microvessels of neurohypophysis were evaluated in a model of the cerebral infarction initiated by a photochemical reaction in the cerebral cortex. After photochemically induced platelet aggregation, we observed the morphological features of angiogenesis.
METHOD: The model of photochemically-induced cerebral ischemia was used. Seven days after intravenous injection of rose bengal and irradiation from a halogen lamp source through an intact cranium, the sampled material from neurohypophysis is processed for transmission electron microscopy using standard procedures.
RESULTS: We observed morphological features of the new vessel formation: the alterations in the endothelium and extracellular matrix during separation of the endothelial cell from each other in a "mother" vessel, the migration of the endothelial cells in the extracellular matrix, the communication of the lumen of the new and the "mother" vessels.
CONCLUSION: We observed development of the angiogenic phenotype in the neurohypophysis after focal ischemia in the cerebral cortex. The endothelium, basement membrane and extracellular matrix undergo morphological alterations which lead to new blood vessel formation.

Introduction

Angiogenesis involves the most dynamic functions of the endothelium. In response to an angiogenic stimulus, endothelial cells in a "mother" vessel separate from each other, leaving uncovered segments of the basement membrane, and migrate. Other endothelial cells divide. Migrating and proliferating cells form loops and then tubes. Basement membrane is secreted to cover the sprouts, and the lumen of these tubes communicates with that of the "mother" vessel. This formation of sprouts continues until the necessary microvascular network is formed [1].

Whereas the role of new blood vessel formation in cancer [2, 3], and development [4], and wound healing [5] has been well documented, the use of angiogenesis to restore blood flow of ischemic brain is a novel form of therapy preventing further disease progression.

In our studies we observed development of the angiogenic phenotype in the neurohypophysis after focal ischemia in the cerebral cortex. The endothelium and extracellular matrix undergo morphological alterations which lead to new blood vessel formation.

Material and methods

Sixteen male Wistar rats (150–200 g) were divided into two experimental groups. The first group was anaesthetized with 325 mg/kg chloral hydrate and injected intravenously with a 3% solution of rose bengal (Sigma. St. Louis, USA) in physiological saline at a dose of 40 mg/kg. The animals from the control group were injected with rose bengal but not irradiated. The photochemical reaction was performed as described previously [4]. Briefly, the skin was incised to the parietal bone, the periostium was removed and the animals were irradiated through the scull over the left hemisphere with a hallogen light bulb for 30 min. Material for microscopic studies was sampled from neurohypophysis 7 days after irradiation in the experimental group and 7 days after injection of rose bengal in the control group. The animals were anaesthetized with ether and perfused with 2,5% glutaraldehyde in cacodylate buffer. The sampled material was processed for transmission electron microscopy using standard procedures.

Results

Material from the control group, that was not irradiated was ultrastructurally unchanged. The morphological features of angiogenesis were usually not observed.

Seven days after ischemia of the cerebral cortex in sections from the neurohypophysis, morphological features of angiogenesis were present. In Fig. 1 the separation of endothelial cells from each other in a mother vessel is seen. The basement membrane of the mother vessel is blurred and extracellular matrix surrounding it is homogenous and consists of the fine fibrillar material devoid of collagen fibrils. In the altered extracellular matrix there is also, probably a new formed capillary vessel showing high endothelial cells and narrow lumen. In the next step, the migration of endothelial cells is present (Fig. 2). The endothelial cells migrate one by one in homogenous extracellular matrix. Migrating cells partly connected by junctions are visible among the neurohypophysis cells where they are accompanied by perivascular cells.

Communication of the new formed capillary with the mother vessel is present in Fig.3. Endothelial cells of the young vessel are high and partly connected by junctions. Between the endothelial cells of the young vessel we observe part of endothelium from the mother vessel. The extracellular matrix surrounding connected young and mother vessels is morphologically unchanged.

Discussion

Angiogenesis is involved in many normal and pathologic conditions including wound healing, tissue graft, embryogenesis, inflammation, rheumatoid arthritis and neoplasia. Normal neovascularization is necessary for uncompromised wound healing and tissue regeneration, and therefore the angiogenic process often becomes the target for treatment of impaired wounds or tissue grafts.

The development of the angiogenic features is a complex process and involves the ability of the endothelial cell to break homotypic cell contacts, migrate through basement membrane and extracellular matrix, proliferate and reorganize to give an intact neovessel with a patent lumen. The morphological features of all these steps were presented in our microscopic studies. The changes observed in endothelial cells during new blood vessel formation were accompanied by alterations of basement membrane and extracellular matrix. It is well known that structural changes in the extracellular matrix are necessary for cell migration during tissue remodelling, tumor invasion and wound healing [7]. Angiogenesis is a complex process involving endothelium changes as well as extracellular matrix synthesis and differentiation [8]. In these processes many classes of agents may promote necessary degradation of extracellular matrix [9]. We suppose that morphologically changed extracellular matrix occurs during the activity of these agents.

We observed the correlation between capillary proliferation in neurohypophysis and photochemically-induced ischemia in the cerebral cortex. It was found

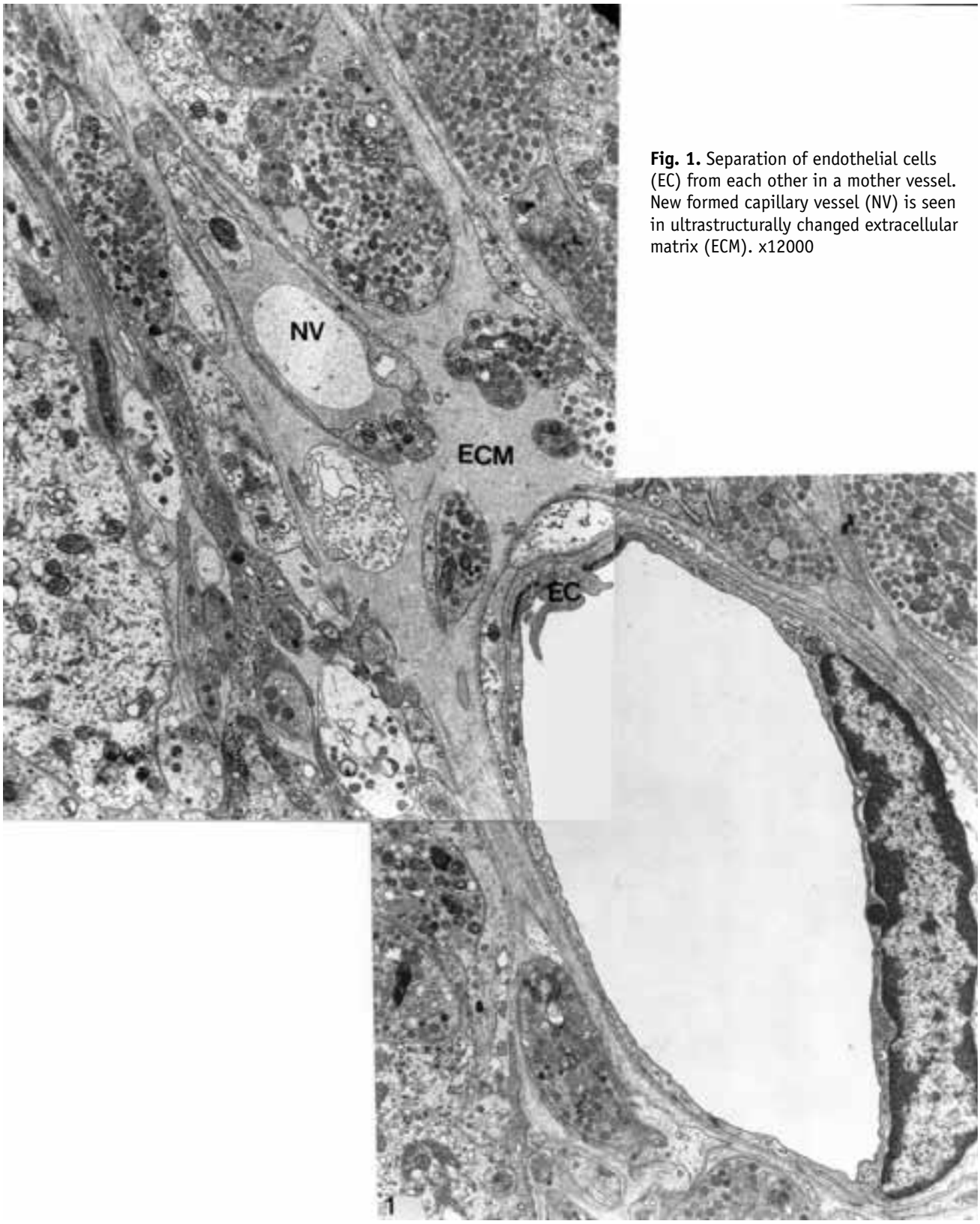

Fig. 1. Separation of endothelial cells (EC) from each other in a mother vessel. New formed capillary vessel (NV) is seen in ultrastructurally changed extracellular matrix (ECM). x12000

that angiogenesis occurs in brain in response to pathologic stimuli such as ischemic injury [10, 11]. Prolonged hypoxia induces systemic and local adaptive responses to improve tissue oxygen delivery, especially to the brain [12, 13]. The adaptive response is the increased brain capillary density which results in shorted intercapillary diffusional distances, thus improving brain tissue oxygenation [14, 15]. Ischemic brain tissues possess angiogenic activity, with large numbers of proliferating endothelial cells in the penumbra [16]. Brain angiogenesis is a tightly controlled

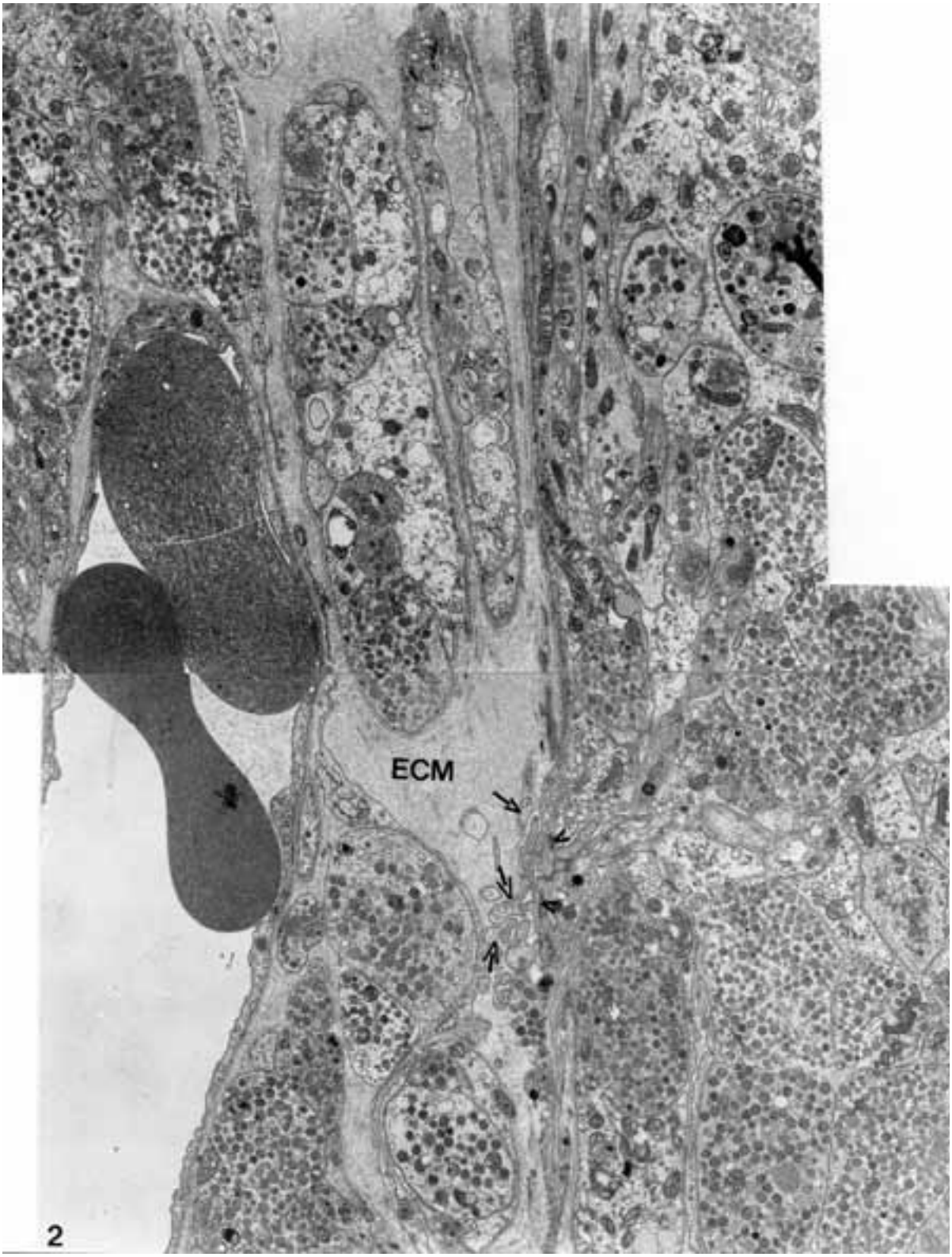

Fig. 2. Migration of endothelial cells (arrows) in ultrastructurally changed ECM. Connections between migrating endothelial cells (arrow heads) are seen. Migrating endothelial cells are accompanied by perivascular cell (asterisk). x15000

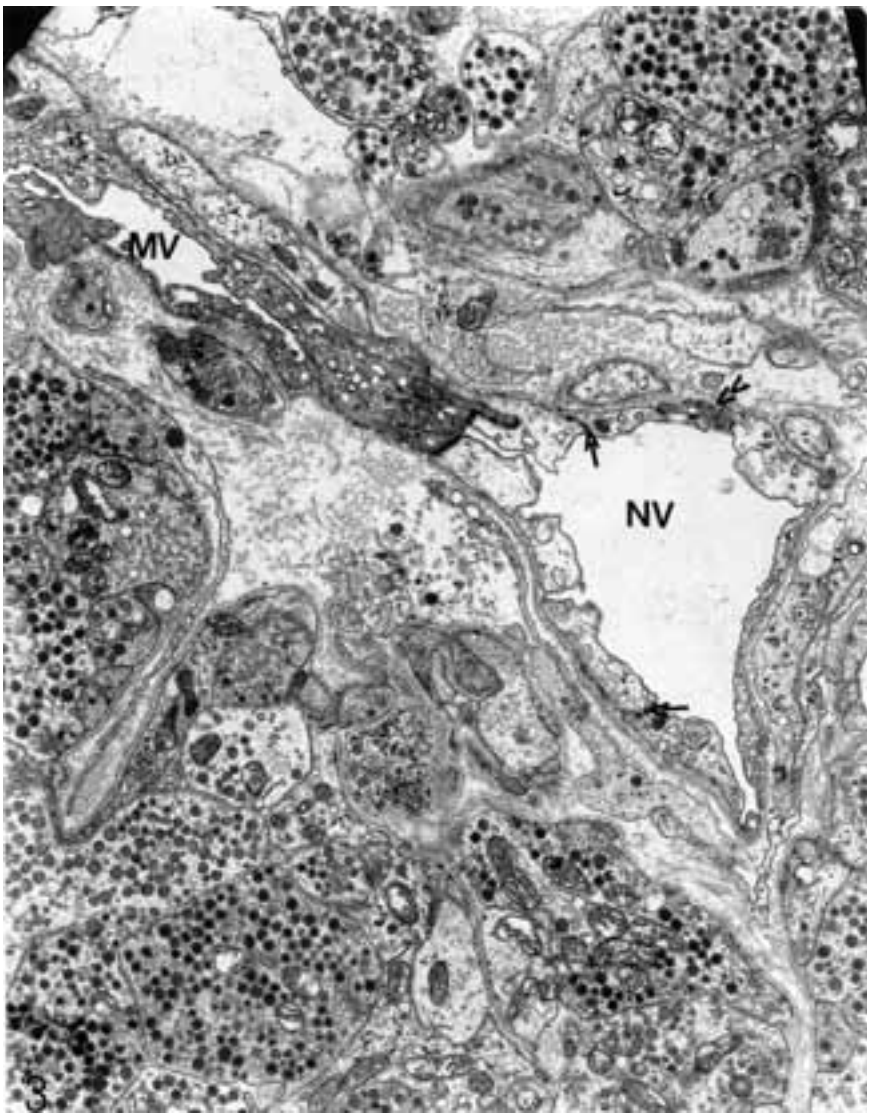

Fig. 3. Communication of the new formed capillary vessel (NV) with mother vessel (MV). Connections between young endothelial cells are seen (arrows). Among young endothelial cells, the part of endothelium from the mother vessel is present (double arrow). x10000

process that is regulated by neuroectodermal derived growth factors that bind to receptors linked with tyrosine kinases expressed on endothelial cells. Current evidence suggests that physiological angiogenesis in the brain is regulated by similar mechanisms as pathological angiogenesis induced by tumors or by hypoxia/ischemia [17, 18]. Neurohypophysial angiogenesis observed in our material is probably induced by a release of the some angiogenic factors from the ischemic focus in the cerebral cortex. Many factors are known to induce angiogenesis, and these include a variety of polypeptide growth factors [19]. Among the polypeptide angiogenic factors, vascular endothelial growth factor (VEGF) has gained much attention because it is a specific mitogen for endothelial cells [20]. In rat brain, two isoforms VEGF are induced by focal ischemia [21, 22]. Therefore, these isoforms may play more important roles in ischemic brains and may induce angiogenesis in neurohypophysis.

Thinking over the reason of effects of the focal cerebral cortical ischemia induced by photochemical reaction on the angiogenesis in neurohypophysis, we have also taken another possibility into consideration. On days one and four after irradiation we observe the thrombi in the neurohypophysial vessels. We should pay attention to a release of angiogenic factors directly from platelets which aggregate in vessels. It is well known that on activation, platelets release their contents including angiogenic growth factors, like VEGF and others [23]. Recently, sphingosine 1-phosphate was reported to be a bioactive lipid released from activated platelets and to interact with endothelial cells under the conditions in which critical platelet-endothelial interactions (including thrombosis and angiogenesis) occur [24].

The process of angiogenesis induced in neurohypophysis by thrombosis of cortical vessels may be beneficial for the tissue under pathologic conditions. A number of studies have demonstrated the key role of angiogenesis for the successful restoring of blood flow in the brain after ischemia [25]. We think that more intensive investigation of angiogenic morpho-

logic phenotype with immunocytochemical studies will be an inexhaustible source of information about this process.

REFERENCES

1 Furcht LT. Critical factors controlling angiogenesis: cell products, cell matrix and growth factors. Lab Invest 1986; **55**:505–509.
2 Fidler I J, Ellis LM. The implication of angiogenesis for the biology and therapy of cancer metastasis. Cell 1994; **79**:185–188.
3 Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other diseases. Nature Med 1995; **1**:27–31.
4 Risau W, Flamme I. Vasculogenesis. Ann Rev Cell Dev Biol 1995; **11**:79–91.
5 Hockel M, Shlenger K, Doctrow S, Kissel T, Vaupel P. Therapeutic angiogenesis. Arch Surg 1993; **128**:423.
6 Gajkowska B, Frontczak-Baniewicz M, Gadamski R, Barskov I. Photochemically-induced vascular damage in brain cortex. Transmission and scanning electron microscopy study. Acta Neurobiol Exp 1997; **57**:3–208.
7 Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. Induction of cell migration by matrix metalloproteinase-2 cleavage of laminin-5. Science 1997; **277** :225–228.
8 Folkman J. Angiogenesis:initiation and control. Ann NY Acad Sci 1982; 212–227.
9 Alexander CM, Werb Z. Extracellular matrix degradation. In: Cell Biology of Extracellular Matrix, Hay ED, editor. New York: Plenum Press; 1991. pp. 255–302.
10 Du Bois M, Bowman PD, Goldstein GW. Cellular proliferation following ischemic injury in the gerbil brain. J Cell Biol 1983; **97**:246a.
11 Krupinski J, Kaluza J, Kumar P, Kumar S, Wang JM. Role of angiogenesis in patients with cerebral ischemic stroke. Stroke 1994; **25**:1794–1798.
12 Cervos Navarro J, Sampaolo S, Hamdorf G. Brain changes in experimental chronic hypoxia. Exp Pathol 1991; 42:205–212.
13 Monge C, Leon Velarde F. Physiological adaptation to high altitude: oxygen transport in mammals and birds. Physiol Rev 1991; **71**:1135–1172.
14 Harik N, Harik SI, La Manna JC. On the increased cerebral vascularity in hypobaric hypoxia: time course and reversibility in adults rats. J Cereb Blood Flow Metab 1995; **15**:S276.
15 La Manna JC, Vendel LM, Farrel RM. Brain Adaptation to chronic hypobaric hypoxia in rats. J Appl Physiol 1992; **72**:2238–2243.
16 Krupinski J, Kumar P, Kumar S, Kaluza J. Increased expression of TGF-beta 1 in brain tissue after ischemic stroke in humans. Stroke 1996; **27**:852–857.
17 Plate KH: Mechanisms of angiogenesis in the brain. J Neuropathol, Exp Neurol 1999; **58**:313–320.
18 Marti HJ, Bernaudin M, Bellail A, Schoch H, Euler M, Petit E, Risau W. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol 2000; **156**:965–976.
19 Yoshimoto T, Houkin K, Takahashi A, Abe H. Angiogenic factors in moyamoya disease. Stroke 1996; **27**:2160–2165.
20 Wang W, Merril MJ, Borchardt RT. Vascular endothelial growth factor affects permeability of brain microvessel endothelial cells in vitro. Am J Physiol 1996; **271**:C1973–C1980.
21 Hayashi T, Abe K, Suzuki H, Itoyama Y. Rapid induction of VEGF gene expression after transient MCA occlusion in rat. Stroke 1997; **28**:2039–2044.
22 Kovacs Z, Ikezaki K Samoto K, Inamura T, Fukui M. VEGF and flt. Expression time kinetics in rat brain infarct. Stroke 1996; **27**:1865–1873.
23 Verheul HMW, Jorna AS, Hoekman K, Broxterman HJ, Gebbink MFBG, Pinedo HM. Vascular endothelial growth factor-stimulated endothelial cells promote adhesion and activation of platelets. Blood 2000; **96**:4216–4221.
24 Yatomi Y, Ohmori T, Rile G, Kazama F, Okamoto H, Sano T, Kaneo S, Kume S, Tigyi G, Igarashi Y, Ozaki Y. Sphingosine 1-phosphate as a major bioactive lysophospholipid that is released from platelets and interacts with endothelial cells. Blood 2000; **96**:3431–3438.
25 Szpak GM, Lechowicz W, Lewandowska E, Bertrand E, Wierzba-Bobrowicz T, Dymecki J. Border zone neovascularization in cerebral ischemic infarct. Folia Neuropathol 1999; **37**:264–268.