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

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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 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...
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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 process.
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
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