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

I. Ultrastructural changes in capillary vessels of the neurohypophysis after focal ischemia of the cerebral cortex

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Abstract

OBJECTIVES: In our investigations we have reported that photochemical reaction leading to brain ischemia can also be precipitated with visible light from a non-coherent light source. It was revealed that focal cerebral ischemia after photochemical reaction cause the alterations in the capillaries ultrastructure and perivascular spaces of the barrier-competent regions of the brain. The purpose of this study is to first characterize the ultrastructural morphological consequences of photochemically induced ischemia in the cerebral cortex on the capillaries of neurohypophysis as the barrier-free region of the brain.

METHOD: We used a model of ischemic brain damage due to obliteration of microvessels following the photochemical reaction. Rats were treated with an intravenous injection of rose bengal and irradiated from a halogen lamp source through an intact cranium to precipitate microvascular damage. Material for electron microscopic studies were sampled from the neurohypophysis 1 and 4 days after irradiation (4 animals in each group) in experimental group and 1 and 4 days after a rose bengal injection in control group.

RESULTS: Investigations in transmission electron microscopy revealed platelet aggregation on the endothelium preceded by its early ultrastructural damage. In the capillaries of the neurohypophysis, one and four days after irradiation, numerous microthrombi adhering to the damaged endothelium were present. The capillary vessels contained a continuous, rather than a fenestrated endothelium. The basement membrane was thickened, blurred and locally multiplicated.

CONCLUSION: Our results show that experimentally-induced thrombosis of cortical microvessels leads to alterations in the capillaries of neurohypophysis.
**Introduction**

Research on pathogenesis and mechanisms of cerebral ischemia relies on the availability of suitable experimental models. Watson et al. [1] have shown that brain ischemia can be experimentally induced by an intravascular photochemical reaction causing thrombosis in cortical vessels. The animals were injected with a dye, rose bengal, which initiated the thrombotic reaction upon irradiation with laser light through an intact skull.

Our studies revealed that focal cerebral ischemia after photochemical reaction causes alterations in the capillaries ultrastructure and perivascular spaces of the barrier-competent and barrier-free regions of the brain.

Brain endothelium is unique among other organs because it forms the highly impermeable blood-brain barrier. The microvessels forming the blood-brain barrier are constituted of non-fenestrated endothelial cells connected by tight junctions. However, some regions in the brain such as area postrema, choroid plexus, pituitary and pineal gland, subfornical organ and median eminence, require fenestrated or permeable endothelium for their function [2, 3]. In the neurohypophysis, the tight junctions between endothelial cells are absent and the endothelial layer is not continuous.

Our previous studies on capillaries of the cerebral cortex [4] revealed platelet aggregation on endothelial cells preceded by its early ultrastructural damage. One and four days after irradiation, deep invaginations on the endothelial surface were present and the interendothelial junctions were elongated. We observed shrinkage and sloughing of the endothelial cells and an increase in the number of cytoplasmic microvesicles in capillaries of the cerebral cortex. The purpose of this study is first to characterize the ultrastructural morphological consequences of photochemically induced ischemia in the cerebral cortex on the capillaries of neurohypophysis.

**Material and methods**

Male Wistar rats (150–200 g) were used. Eight animals were anaesthetized with 325 mg/kg chloral hydrate and injected intravenously with rose bengal (Sigma, St Louis, USA) in physiological saline at a dose of 40 mg/kg. Another 8 animals were injected with rose bengal but not irradiated and used as controls. The head was immobilized in the stereotactic apparatus, the skin was incised in the sagittal plane to the parietal bone. The periosteum was removed and the brains of both experimental and control animals were irradiated through the intact skull over the left hemisphere, half distance between the coronal and lambdoid sutures. An air-cooled halogen light bulb of 250 W (Osram) was used as a light source. Light was transmitted to the skull with an optical wave-guide, the ending of which was placed over the preselected part of bone. The skull-light pipe interface was cooled by water to remove the thermal effect irradiation. After a 30 min irradiation session the animals were housed in standard conditions. Material for microscopic studies were sampled from the neurohypophysis 1 and 4 days after irradiation (4 animals in each group) in experimental group and 1 and 4 days after a rose bengal injection 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**

Control neurohypophysis contained fenestrated endothelium in the capillaries. The endothelial cells had electron lucent cytoplasm with morphologically unchanged organelles. There were no tight junctions between adjacent endothelial cells and the very

![Ultrastructurally unchanged capillary vessel with fenestrated endothelium, without tight junctions between adjacent endothelial cells (arrows) and very thin basement membrane (BM). Extracellular matrix (ECM) is well developed. x12000](image)
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**Fig. 2.** One day after photochemical reaction. Capillary vessel with erythrocytes (E) and thrombocytes (T) in the lumen. x6000

**Fig. 3.** One day after irradiation. Erythrocytes adhere to the endothelium. Basement membrane (BM) and extracellular matrix (ECM) are ultrastructurally unchanged. x6000
Fig. 4. Four days after irradiation. Capillary vessel with continuous rather than fenestrated endothelium. Thrombus adhere to partially lacking plasma membrane (arrow). The BM is thickened and locally goes deep into perivascular cell (asterisk). x12000

Fig. 5. Four days after photo-chemical reaction. Part of capillary vessel with continuous endothelium. Numerous microvesicles (arrows) are present along the thickened and blurred BM. Between the multiplied BM swollen the perivascular cell is present (asterisk). x24000
thin basement membrane was occasionally uncovered (Fig. 1). The extracellular matrix was well developed and contained large quantities of collagen. One day after photochemical reaction numerous microthrombi consisting of thrombocytes and erythrocytes were present in the microvessels (Fig. 2, 3). Basement membrane and extracellular matrix were ultrastructurally unchanged. In some capillary vessels, thrombocytes and erythrocytes constituting the thrombus adhered to the endothelium. The endothelial cells were partially lacking plasma membrane on the luminal surface, then thrombus adhered directly to the damaged endothelium as it was observed four days after irradiation. These vessels contained a continuous rather than a fenestrated endothelium with a smooth luminal surface (Fig. 4). In the electron-lucent cytoplasm of the endothelial cells, numerous microvesicles were found along the basement membrane. The basement membrane was thickened, blurred and locally multiplicated. Sometimes, thickened basement membrane went deep into perivascular cells. Between the multiplicated basement membrane swollen perivascular cells were present (Fig. 5). The collagen containing extracellular matrix was ultrastructurally unchanged when compared to the control.

Discussion

In this paper, examples were presented in which perturbations of the central nervous system capillaries after focal cerebral ischemia in the cortex were closely linked with pathophysiology of microvascular endothelium in neurohypophysis. The main finding of this study is that a focal cerebral ischemia in brain cortex induces morphological changes in capillaries of neurohypophysis.

Ultrastructural changes were present mainly in endothelial cells and basement membrane and comprised features of endothelial cell damage and thrombus formation. A similar pattern was previously observed in the cerebral cortex [4]. We speculate that light-excited rose bengal dye molecules induce a direct damage in the endothelial cells of the cerebral cortex. Formation of microthrombi is regarded as an early phenomenon responsible for progression of ischemic brain damage [1, 5]. Four days after focal cerebral ischemia the reaction of basement membrane and perivascular cells was seen. We observed swelling of the perivascular cells between multiplicated basement membrane. That morphological feature of basement membrane is probably a consequence of extracellular matrix proteinases activity [6, 7, 8]. The participation of extracellular matrix proteinases was documented in brain after ischemia.

Rosenberg [9] proposed that release and activation of type IV collagenase in injured brain tissue disrupts the blood-brain barrier by attacking collagen in the basal lamina with subsequent necrosis, hemorrhage and migration of white blood cells to the site of injury. In the normal tissues the extracellular matrix is protected from collagenolytic attack because of multilevel regulation of enzyme activity. The first level is the activation of proenzyme which can be activated by several mechanisms including free radicals [10]. The free radicals released during irradiation and activation of the rose bengal in our experiment are probably responsible for initiation of proenzymy activity. The endogenous mechanisms for the activation of collagenase are unclear. Potential activating agents are present in the brain or can be bought into the brain along with invading cells. In cerebral ischemia, hemorrhagic conversion of the infarcted region and secondary opening of the blood-brain barrier occur as late sequelae of the injury.

We observe the capillaries of a continuous, nonfenestrated endothelium. These capillaries may be an example of a newly regenerated vessel in the neurohypophysis. We suggest, that the modification of the endothelium features in the neurohypophysis is connected with remodelling of the cytoskeleton of the endothelial cell [11]. Residing at the interface between the circulation and the brain parenchyma, the microvascular endothelium of the central nervous system is uniquely positioned to be a critical regulator of CNS function. Therefore, it should come as no great surprise that disturbances to this vascular tissue might exacerbate or play roles in several neurological conditions. As such, altered functioning of this boundary can incite a reverberating cascade of cytopathic activity that reaps destruction through the CNS to other parts of the brain.

The relationship between focal cerebral ischemia in the cerebral cortex and changes in capillaries of the neurohypophysis is a very interesting clinical problem. The capillaries of the neurohypophysis play an important part in connection between the hypothalamus and hypophysis. To deliberate about the reason of this phenomenon we have taken into consideration the cerebrovascular permeability of cortical vessels in relation to the development of brain edema in a photochemical lesion [12]. The fact that we demonstrated alterations of neurohypophysial vessels after cortical infarction is probably not due to extravasations in situ but due to transport of protein by bulk flow from the lesions. It is possible that the changes in sodium permeability or other small solutes mediated by the release of vasoactive substances [13] may evoke these neurohypophysial changes. It is also possible that this is the direct effect of the photochemically modified
molecules of the photosensitive dye on the neurohypophysial vessels. Such molecules could possibly penetrate in the neurohypophysial region.

Mechanical disruption to the vessel wall owing to shear stress during an abrupt cessation of cerebral blood flow is another possibility of this relationship. The flow of blood imposes a fluid shear stress on the endothelial cells and studies have demonstrated that it plays an important role in modulating the structure and function of these cells [14]. A number of studies have demonstrated that the function and morphology of the vasculature are regulated by hemodynamic stress [15]. Wall shear stress is a rheological force that shears the luminal surface of the blood vessel when blood flows over the endothelial wall. Shear stress induces an endothelial cell membrane deformation and cytoskeletal rearrangement, which transfers stress to different regions of the endothelial cell where mechanotransduction may occur.

In summary, after experimentally induced thrombosis of cortical microvessels we observe alterations in capillaries of the neurohypophysis. Whether similar alterations are present in patients during a thrombotic event is an obvious but unanswered question. It therefore seems justified that the functional consequences of vascular thrombosis should be explored experimentally.

REFERENCES