Physiological and Morphometric Analysis of the Microcirculation of the Cerebral Cortex Under Acute Vasospasm

N. Wiernsperger, Ph.D., U. Schulz, M. Sc., P. Gygax, Ph.D.

SUMMARY  Tissue Po2 measurements with microelectrodes and morphometric analysis of capillaries were combined to investigate microcirculatory changes in cat brains subjected to vasospasm (superfusion of platelet-poor plasma). It can be shown that vasospasm leads to marked tissue hypoxia as illustrated by a drastic shift in the Po2 distribution within the cortical tissue. Morphological measurements of various capillary parameters demonstrated a marked constriction in precapillary segments as well as in capillaries themselves. This decrease in perfusion of the capillary bed could not be compensated for by increasing the systemic arterial blood pressure.

Our results demonstrate that, even with a platelet-poor fraction, marked changes occur on a microcirculatory level in the cerebral cortex with vasospasm, which may not be detected by macroscopic techniques.

VASOSPASM can be observed after subarachnoid hemorrhage, mechanical trauma of vessels or even in the late phases of ischemia. Its high frequency and the lack of an effective therapy have made vasospasm and its complications a serious problem in neurosurgery. The literature dealing with the physiological or morphological aspects of vasospasm mainly concerns the larger brain vessels, neglecting the consequences of spasm on the microvasculature. Tissue oxygen measurements with morphometric analysis of the capillary system were made to investigate the effects of acute vasospasm on the microcirculation of the cerebral cortex.

Methods

The experiments were performed on 8 adult cats (n = 4 in both control and with vasospasm animals), anesthetized with N2O/O2 (70%, 30% respectively) and, after paralysis with Flaxedil, artificially ventilated. The body temperature was maintained at 37°C throughout the experiment. The arterial blood pressure, heart rate and end-expiratory PCO2 were monitored. Animals where these measurements varied significantly from normal were disregarded.

Using a trepane, a hole was made in the skull over the parietal cortex and the dura removed. Platelet-poor plasma was obtained by centrifugating 5 ml of fresh blood from the same animal at 4500 RPM for 10 min. The supernatant plasma was then decanted into a fresh blood from the same animal at 4500 RPM for 10 min. The supernatant plasma was then descanted into a glucose-glucose oxidase solution and PO2 = 0 was determined by dipping the electrode in a glucose-glucose oxidase solution and PO2 = max in air-equilibrated physiological saline at 37°C. A large number of individual Po2 values were recorded by moving the electrode over the area of interest. All values were grouped into classes of 10 mm Hg and plotted on a histogram, thus showing the distribution of oxygen within the superfused tissue area.

At the end of the experiment, the animal was killed and the skull opened further. The suprasylvian gyrus, where Po2 measurements had been made, was extracted. Slices 0.5 cm thick were cut and immediately frozen in petroleum ether-dry ice. For histological processing, 14 μm thick serial sections were cut using a cryostat. The capillaries were then stained by the alkaline phosphatase (aPs) reaction for analysis with an optical-electronic image analyzer, the Leitz-Classimat.

From each brain, a total of 75 measuring fields (each test field = 113.800 mm³) were analyzed within the suprasylvian cortex. Including the average section thickness of 14 μm, the total test volume was 1.2 × 10⁶ μm³ per brain.

In our laboratory, the Classimat is on-line with a computer. This permits transfer of picture coordinates from the television system directly to computer by an interface and digitalization of the whole television picture. Using this method, various parameters of single capillary fragments can be determined: diameter D (μm), volume fraction VV (%) surface-to-volume ratio S/V (μm⁻¹), mean minimal distance ΔxMB (μm) between capillary centers of gravity, length per unit volume LHV (cm/mm³) and number of capillary fragments per test area NIAL.

Capillaries (φ 3–8 μm) and vessels belonging to the precapillary system (φ 9–14 μm) were classified according to their diameter. A total of 6000 capillary fragments were counted and measured for calculating average values of stereological parameters in each experimental group. Finally, statistical analysis were...
Physiology

The macroscopic observation of the brain surface at the end of the vasospasm induction period revealed only slight blanching of the cortical tissue. However, the vasospastic state of cerebral vessels resulted in a marked reduction in tissue $pO_2$, as indicated by the dramatic increase in the frequency of low $pO_2$ values in the histogram, indicative of tissue hypoxia (fig. 1). Higher $pO_2$ values completely disappeared. The mathematical center of gravity of the $pO_2$ histogram was shifted from 29.8 torr in control animals to 19.7 torr after vasospasm. The model used showed also a good reproducibility, as all animals exhibited the same effects on tissue $pO_2$.

To determine if an increase in cerebral perfusion pressure would overcome the constriction due to vasospasm and reestablish a better oxygen supply to the tissue, the mean arterial blood pressure was elevated by a slow intravenous infusion of a sympathomimetic drug (Etilerine, Effortil, Boehringer). In control animals, this resulted in a slight, progressive increase in tissue $pO_2$. The reaction of individual $pO_2$ measuring sites with vasospasm was different according to electrode location. An example is given in figure 2 which shows the reaction to an increase in blood pressure in 3 (of numerous) tissue $pO_2$ measurements. In the vasospastic group, the blood pressure increase led to either stable, elevated or decreased oxygenation of the cortical tissue. This divergent reaction is characteristic for shunt perfusion, illustrating a patchy distribution of flow in the cortical microcirculation. Analogous results have been obtained by using Rheomacrodex as a vascular volume surcharge for increasing the systemic blood pressure. The development of brain edema could be observed when blood pressures of 180 mm Hg were reached.

Morphometry

Statistical comparison of stereological capillary measurements from the suprasylvian gyrus of the control and vasospastic groups is shown in the table. The results, as expected from the tissue $pO_2$ measurements, show a distinct alteration of the capillary system after induction of vasospasm. Compared with the control animals, a highly significant $(p < 0.0025)$ diminution of the capillary diameter $D_c$ from 5.4 $\mu$m to 4.3 $\mu$m occurred after vasospasm, the volume fraction $V_{vt}$ decreased significantly $(p < 0.01)$ as well as the total capillary length per unit cortex volume $L_{vt}$ $(p < 0.05)$.

- Figure 1. $pO_2$-histograms indicating the distribution of oxygen within the cerebral cortex of control ($n = 4$) cats and animals submitted to acute vasospasm ($n = 4$). Each histogram consists of 120 $pO_2$ values. The arrow indicates the center of gravity of the histogram.
- Figure 2. Change of tissue $pO_2$ to a systemic blood pressure increase in control and spastic animals. Three representative $pO_2$ are illustrated here.
TABLE

Comparison Between Controls and Animals with Vasospasm (t-value limit of significance: \( p < 0.05 \)).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vasospasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>( D_1 (\mu m) )</td>
<td>5.35 ± 1.01</td>
<td>4.26 ± 0.36</td>
</tr>
<tr>
<td>( V_l (%) )</td>
<td>4.69 ± 1.27</td>
<td>2.72 ± 0.11</td>
</tr>
<tr>
<td>( S_l/V_l (\mu m) )</td>
<td>0.55 ± 0.05</td>
<td>0.67 ± 0.06</td>
</tr>
<tr>
<td>( \Delta A_B \mu m )</td>
<td>±4.95 ± 0.05</td>
<td>±5.46 ± 0.05</td>
</tr>
<tr>
<td>( L_{vi} \text{cm/mg} )</td>
<td>43.00 ± 0.14</td>
<td>43.00 ± 0.01</td>
</tr>
<tr>
<td>( N_i/AT )</td>
<td>45.53 ± 2.21</td>
<td>44.69 ± 1.11</td>
</tr>
</tbody>
</table>

\( i = \) index of capillaries; \( D_1 = \) diameter; \( V_l = \) volume fraction; \( S_l/V_l = \) specific surface area; \( \Delta A_B = \) minimal intercapillary distance; \( L_{vi} = \) total length per unit cortex volume; \( N_i/AT = \) number of capillary fragments per test area.

In consequence, the surface-to-volume ratio \( S_l/V_l \) increased \(( p < 0.0025 \)). The minimal intercapillary distance \( \Delta A_B \) and, accordingly, the number of capillary fragments \( N_i/AT \), revealed no changes after induction of vasospasm.

**Discussion**

Several fractions of fresh blood have been demonstrated to induce vasospasm. Despite the fact that we utilized platelet-poor plasma, which is known to have the least effect of all blood fractions, the vasospasm was sufficient to induce a very marked hypoxia within cortical tissue. The reduction in oxygen availability correlates well with similar observations concerning cerebral blood flow measurements under vasospastic conditions. The \( P_o \) histogram obtained with vasospasm closely resembles the distribution observed in another model of cerebrovascular insufficiency, namely hypovolemic oligemia. It is of special interest to note that our findings of a reduced tissue oxygenation and constriction of capillaries are found immediately after induction of vasospasm and at a time when the brain surface appears normal.

The strong vasospastic state of the vessels is further demonstrated by the inability of an increased perfusion pressure to overcome the vascular contraction. Under such conditions, the blood vessels offer enough resistance to force the blood to preferentially perfuse the vascular pathways of least resistance, thus leading to a maldistribution in the microcirculation. It was reported by Jakubowski et al. that the maintenance of autoregulation of cerebral vessels followed a rise in blood pressure under vasospastic conditions, an observation based on cerebral blood flow and radiological measurements, which involved large areas of brain tissue. Our results, obtained with the \( P_o \) micro-electrode technique, which has a high resolution, demonstrate that although the global cerebral blood flow may be the same, the microcirculation is strongly impaired under conditions of vasospasm at the time of an increase in perfusion pressure. A blood pressure increase over the high limit of autoregulation leads to rapid development of edema in vasospastic vessels.

Vasospastic states induced by electrical stimulation of the brain have shown that the intraparenchymal arterioles, but not the capillaries, exhibit constriction. Our morphometric results indicate that on a microcirculatory level a vasospastic state of smaller vessels, including true capillaries, exists which may not be detected by angiographic techniques used in clinics. Also, the stereological investigation of the capillary network indicates changes with vasospasm which are as marked as those seen under conditions of oligemia.

The application of plasma (or physiological saline in control animals) to the brain surface was performed in such a way that mechanical stimulation of the pial vessels was unlikely to occur. Therefore, even platelet-poor plasma contains a spasmogenic factor potent enough to cause vessel constriction.

The main question arising from this study is whether the capillary constriction is due to active or passive mechanisms. The reduction in capillary diameter can be passive due to the fall in perfusion pressure gradient distal to the spasm of arterial or arteriolar vessels. This would limit the amount of blood and oxygen available for the capillary bed. The decrease in capillary diameter could interfere with a normal passage of erythrocytes through the vessel lumen and modify the oxygen supply to the tissue.

Another explanation is that the spasmogenic factor leads directly to an active constriction of the capillaries. These microvessels contain actin and myosin filaments, which suggests that they are capable of constricting. Specialized endothelial cells lie at the capillary branchings and along the vessels and are innervated and capable of swelling, thus reducing the lumen of the vessel. Though the latter results originate from non-cerebral capillaries, there is evidence supporting the innervation and contractile capacities of cerebral capillaries.

This study shows that a combination of physiological and quantitative morphological methods using the same material yields precise information about the pathophysiology of microcirculation, and
allowed demonstration of marked microcirculatory changes occurring during vasospasm which cannot be detected by macroscopic techniques.

References

Transient Ischemic Attacks and External Carotid Artery
A Retrospective Study of 23 Patients with an Occlusion of the Internal Carotid Artery

Julien Bogousslavsky, M.D., Franco Regli, M.D., Jean-Pierre Hungerböhler, M.D., and Richard Chrzanowski, M.D.

SUMMARY Twenty-three patients with occlusion of an internal carotid artery have been followed 5 to 60 months after angiography. None had a later permanent stroke. Eight had delayed TIAs in the occluded internal carotid area, never in another area. In these TIAs the role of the homolateral external carotid artery is emphasized, because in the 8 cases this artery was the main collateral to the occluded internal carotid, and angiography had shown atheromatous stenosis of homolateral external/common carotid arteries or an irregular stump at the site of the occlusion. Hemodynamic and embolic mechanisms are discussed, especially the latter, because of the absence of severe stenosis and evidence of emboligenic plaques.

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THE ROLE of the external carotid artery in the pathogenesis of some intracranial ischemic events has been reported.1-4 The external carotid artery does not contribute to cerebroretinal perfusion, but when the homolateral internal carotid is obstructed, collateral supply from the external carotid may become important. In this situation, pathological changes in the external carotid artery may have ischemic consequences in the cerebrum or retina through embolic or hemodynamic mechanisms. We studied this situation in 23 patients.

Material and Methods
A retrospective study was made of 23 patients with an angiographically proved occlusion of an internal carotid artery. Five to 60 months after angiography (mean: 27 months) patients were asked about new transient ischemic attacks (TIAs). Angiograms were re-studied (archography and/or selective carotid arteriography). In 11 patients a contralateral carotid arteriogram was made and in 18 a Doppler-carotid ultrasonogram was taken. Eighteen men and 5 women aged 39 to 78 years (mean: 61.5) were studied. Fourteen had a left internal carotid occlusion and 10 a right internal carotid occlusion. In 11 patients a contralateral carotid arteriogram was made and in 18 a Doppler-carotid ultrasonogram was taken. Eighteen men and 5 women aged 39 to 78 years (mean: 61.5) were studied. Fourteen had a left internal carotid occlusion and 10 a right internal carotid occlusion. In 11 patients hospitalization occurred after a minor stroke, and 2 after TIAs. All these ischemic events were in the area of the brain supplied by the occluded internal carotid. Thirteen patients had previously had TIAs in this area, and
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