Acute vasoconstriction: decrease and recovery of cerebral blood flow after various intensities of experimental subarachnoid hemorrhage in rats

Laboratory investigation

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Object. Immediate vasoconstriction after subarachnoid hemorrhage (SAH) has been observed in a number of experimental studies. However, it has not yet been examined which pattern this acute-type vascular reaction follows and whether it correlates with the intensity of SAH. It was the purpose of the present study to vary the extent of SAH using the endovascular filament model of SAH with increasing filament sizes and to compare the course of intracranial pressure (ICP), cerebral perfusion pressure (CPP), and regional cerebral blood flow (rCBF).

Methods. Male Sprague-Dawley rats were subjected to SAH using the endovascular filament model. Subarachnoid hemorrhage was induced using a 3-0, 4-0, or 5-0 Prolene monofilament (8 rats in each group). Eight animals served as controls. Bilateral rCBF (laser Doppler flowmetry), mean arterial blood pressure, and ICP were continuously monitored. Thereafter, the rats were allowed to wake up. Twenty-four hours later, the animals were killed, their brains were removed, and the extent of SAH was determined.

Results. After induction of SAH, ICP steeply increased while CPP and rCBF rapidly declined in all groups. With increasing size of the filament, the increase of ICP and the decrease of CPP were more pronounced. However, the decline of rCBF exceeded the decline of CPP in all SAH groups. In a number of animals with minor SAH, an oscillating pattern of rCBF was observed during induction of SAH and during early recovery.

Conclusions. The disparity between the decline and recovery of CPP and rCBF suggests that acute vasoconstriction occurs even in SAH of a minor extent. Acute vasoconstriction may contribute significantly to a perfusion deficit in the acute stage after SAH. The oscillating pattern of rCBF in the period of early recovery after SAH resembles the pattern of synchronized vasomotion, which has been thoroughly examined for other vascular territories and may yield therapeutic potential. (DOI: 10.3171/2008.8.JNS08591)

Key Words • acute vasoconstriction • cerebral perfusion pressure • intracranial pressure • rat • regional cerebral blood flow • subarachnoid hemorrhage

The phenomenon of acute vasoconstriction after SAH has been observed in several previous animal studies. In 1968, Brawley et al. observed a biphasic pattern of cerebral vasoconstriction with an acute and delayed response after experimental SAH. Echlin performed serial angiographies in monkeys and observed diffuse vasoconstriction immediately after injection of blood into the subarachnoid space. In a rat model of SAH, Bederson and associates observed a discrepancy of CPP and CBF in the first 60 minutes after induction of SAH, indicating the presence of acute vasoconstriction in this early period after SAH. To our knowledge, no systematic study has been performed that further characterizes the nature of acute vasoconstriction after SAH. In particular, it is not known if acute vasoconstriction constitutes a uniform vascular response to the occurrence of SAH or if the extent of it varies with the intensity of SAH.

A variety of experimental models have been used in the past several decades to examine various aspects of SAH. The endovascular filament model appears to be particularly suitable for the examination of the acute stage after experimental SAH. Furthermore, it has previously been shown that the intensity of SAH can be varied by the use of different filament sizes. It was the aim of the present study to characterize the time course of pathophysiological changes in the acute phase after experimental SAH. The main focus of the study was on the course of CPP and its correlation with cortical blood flow.

Abbreviations used in this paper: CBF = cerebral blood flow; CPP = cerebral perfusion pressure; ECA = external carotid artery; ICA = internal carotid artery; ICP = intracranial pressure; LDF = laser Doppler flowmetry; MABP = mean arterial blood pressure; MCA = middle cerebral artery; rCBF = regional CBF; SAH = subarachnoid hemorrhage.
Acute vasoconstriction after experimental SAH

Methods

For the experiments, 36 male Sprague-Dawley rats (weighing 250–300 g each) purchased from Harlan Wickelmann were used. All experiments were approved by the regional authorities and the district government of Bavaria, Germany. At all stages of the experiments the animals were cared for according to institutional guidelines ensuring proper and humane animal care.

Animal Preparation and Monitoring

The animals were anesthetized using 4% isoflurane, orally intubated, and mechanically ventilated with a room air/O₂ mixture to maintain normal arterial blood gases. After induction of anesthesia, isoflurane was reduced to 2.5% for surgical procedures and to 2% from 30 minutes prior to SAH until the end of the monitoring period. Temporalis muscle and rectal probes were used to monitor the rat’s temperature throughout the experiment. A thermostatically regulated, feedback-controlled heating lamp was used to maintain temporalis muscle and rectal temperature at 37°C. The tail artery was cannulated for continuous measurement of MABP and for blood sampling. Arterial blood gases were measured 30 minutes and 5 minutes before, and at hourly intervals after, induction of SAH.

Laser Doppler Flowmetry and ICP Measurement

A 2-channel laser Doppler flowmeter (MBF3D; Moor Instruments) was used for continuous monitoring of rCBF in the area of the cerebral cortex supplied by the MCA. To place the LDF probes, bur holes were drilled 5 mm lateral and 1 mm posterior to the bregma without injury to the dura mater. The animals were then placed supine with the head fixed in a stereotactic frame with ear bars. A rectangularly bent laser Doppler probe was positioned in each bur hole using a micromanipulator.

For ICP measurement, an additional bur hole was drilled over the right frontal cortex. After the animal had been placed supine, an intraparenchymal Camino ICP probe (Integra Neurosciences) was advanced 2 mm into the brain by a third micromanipulator.

Induction of SAH

Subarachnoid hemorrhage was induced by use of the endovascular puncture method. After surgical exposure of the right cervical carotid bifurcation, temporary aneurysm clips were placed on the common carotid artery and ICA. A Prolene filament (Ethicon, Inc.) was inserted into the ECA and fixed with a silk ligature and the temporary clips were removed. After a recovery period of 30 minutes, the filament was advanced into the ICA until ipsilateral laser Doppler flow decreased, indicating that the tip of the filament was at the intracranial bifurcation of the ICA, occluding the origin of the MCA. The filament then was pushed 2–3 mm further for intracranial vessel perforation. The suture was then quickly withdrawn into the ECA to ascertain reperfusion and development of SAH. Subarachnoid hemorrhage was confirmed by a rapid bilateral decrease of LDF and increase of ICP.

Experimental Groups

The rats were randomly assigned to 1 of 4 groups (8 rats in each group): 1) perforation with a 3-0 Prolene filament; 2) perforation with a 4-0 Prolene filament; 3) perforation with a 5-0 Prolene filament; and 4) sham-operated rats. In the latter group, a 3-0 filament was inserted into the ECA and advanced via the ICA until LDF showed an ipsilateral decline, but was not advanced further.

Termination of the Experiment and Quantification of Subarachnoid Blood

At the end of the monitoring period, the ICP probe, laser Doppler probes, and arterial catheter were removed and the wounds were closed using a skin suture. Isoflurane was withdrawn and the animals were allowed to wake up. Twenty-four hours later, the animals were anesthetized again and transcardially perfused with 2% paraformaldehyde, killing the animals. The brain was removed, SAH was confirmed by autopsy, and the amount of subarachnoid blood was quantified using a semiquantitative SAH score following the Fisher scale. The Fisher scale is used clinically for quantification of blood in patients who suffer aneurysmal SAH, according to the following scores: 0 = no blood visible; 1 = traces of blood visible, no firm blood clot; 2 = unilateral clot; 3 = entire basal subarachnoid space filled with blood clot; and 4 = intracerebral hematoma with or without subarachnoid blood.

Thereafter, the brains were embedded in paraffin and cut into 4-µm-thick coronal sections at 400-µm intervals, and the brain slices were stained with cresyl violet. The slices were microscopically scanned for cerebral infarctions (lacunar or territorial) and for the distribution of subarachnoid blood.

Statistical Analysis

Statistical analysis was performed using SPSS statistical software Version 14.0 (SPSS, Inc.). Physiological data for each time point, as well as LDF and ICP data, were analyzed using a 1-way analysis of variance. When multiple comparisons were indicated, a Bonferroni correction was applied. For calculation of correlations between the amount of subarachnoid blood (SAH score) and the relative value of rCBF, a Spearman rank correlation was used. A probability value of < 0.05 was considered statistically significant. Results are presented as means ± standard deviations.

Results

Two animals had to be excluded from the study due to problems during induction of anesthesia. Two animals in the 5-0 group were also excluded from the study and replaced because advancing the filament to cause SAH did not result in characteristic changes of ICP and rCBF.

Physiological Parameters

Arterial blood gases were kept in the normal range and assessed continuously, beginning at the end of surgical preparation, immediately prior to SAH, and in hourly intervals thereafter. There were no significant differences
between the groups regarding pH, partial pressure of CO₂, and partial pressure of O₂.

**Intracranial Pressure, MABP, and CPP**

The course of ICP from 30 minutes prior to SAH until 2 hours after induction of SAH is depicted in Fig. 1A. In the 3-0 and 5-0 groups, elevations of ICP were statistically significant compared with controls at 1 and 5 minutes after SAH. In the 3-0 group, the elevation of ICP was statistically significant compared with the control group and compared with the 4-0 and 5-0 groups throughout the observation period. Secondary to the elevation of ICP, MABP increased (Fig. 1B). The increase was statistically significant in the 3-0 and 4-0 groups at 1 and 5 minutes after SAH. The differences between the SAH groups were not statistically significant.

Cerebral perfusion pressure prior to SAH was 74 ± 15 mm Hg in the control group, 74 ± 14 mm Hg in the 3-0 group, 72 ± 14 mm Hg in the 4-0 group, and 72 ± 10 mm Hg in the 5-0 group. After SAH, CPP declined to a minimum of 40 ± 32 mm Hg, 61 ± 27 mm Hg, and 63 ± 25 mm Hg in the 3-0, 4-0, and 5-0 groups, respectively. Compared with the control group, CPP was only significantly reduced in the 3-0 group 1 minute after SAH. Thereafter, it recovered rapidly and retained a tendency toward lower levels than in the control group, but failed to reach the level of significance throughout the rest of the observation period. In the 4-0 and 5-0 groups, CPP did not decrease significantly compared with controls at any time point of the experiment. There was no significant difference between the SAH groups (Fig. 1C).

**Regional CBF**

Regional CBF was continuously measured from 30 minutes before, until 2 hours after, induction of SAH. The value measured prior to advancing the filament into the circle of Willis was set as the baseline value (100%) for each animal.

Ipsilateral rCBF declined to a minimum of 7 ± 4%, 19 ± 12%, and 32 ± 40% after SAH in the 3-0, 4-0, and 5-0 groups, respectively. Two hours after SAH, ipsilateral rCBF had recovered to 47 ± 40% of baseline in the 3-0 group, to 58 ± 38% of baseline in the 4-0 group, and to 62 ± 44% in the 5-0 group. In all SAH groups, values were significantly decreased compared with the control group from induction of SAH until 120 minutes thereafter (Fig. 2A).

Contralateral rCBF declined to a minimum of 7 ± 6%, 19 ± 11%, and 26 ± 21% after SAH in the 3-0, 4-0, and 5-0 groups, respectively. After 2 hours, it had recovered to 51 ± 43%, 57 ± 28%, and 78 ± 47%. Compared with the control group, values were significantly lower for 120 minutes in the 3-0 group, for 90 minutes in the 4-0 group, and for 60 minutes in the 5-0 group (Fig. 2B).

**Oscillation of rCBF During and Immediately After SAH**

In 2 animals of the 3-0 group, 5 animals in the 4-0 group, and 4 animals in the 5-0 group, we observed an oscillating pattern during the rapid decline of rCBF at the point of induction of SAH and in the first minutes of recovery. Oscillations had a frequency of 6–10 per minute, an amplitude of 10, and 50% of the relative LDF value, and tended to be more pronounced contralateral to the side of vessel perforation (Fig. 3).

**Distribution of Subarachnoid Blood and Microscopic Analysis**

After perfusion fixation and removal of the brain, no traces of blood were found in the subarachnoid space of the control animals. In the 3-0 group, 2 animals had extensive SAH and a small intracerebral hematoma. Two animals had blood spread over the entire basal subarachnoid space. In the remaining 4 animals, a unilateral subarachnoid blood clot was found. In the 4-0 group, 1 animal had the entire basal subarachnoid space filled with blood, 4 had a unilateral blood clot, and 3 had only minor traces of blood. In the 5-0 group, 5 animals had unilateral clots, and 3 animals had only minor traces of blood. No solid blood clots were observed over the convexity of the animals with SAH. There was no correlation between the amount of blood in the basal subarachnoid space and rCBF over both hemispheres. The correlation of SAH score (amount of subarachnoid blood) and relative rCBF over the right (ipsilateral) hemisphere 5 and 120 minutes after induction of SAH is depicted in Fig. 4.

Microscopic examination of perfusion-fixated brain slices showed the effects of the positioning of the ICP probe in the frontal cortex but no other intracerebral lesions. In particular, we did not find any territorial or lacunar infarctions.

**Discussion**

The present experiments were performed to examine 2 aspects of the filament model of SAH: 1) to study whether the intensity of acute vasoconstriction correlates with the intensity of SAH, and 2) to examine whether the decline of CBF and its recovery follows a certain regular pattern that might give information about its pathogenesis and yield therapeutic potential.

In 1995, Bederson et al.3 and Veelken and colleagues28 first published systematic studies using the endovascular filament model of SAH. Major advantages of this model compared with other experimental models of SAH are that: 1) the intracranial vault and the arachnoid layer do not have to be opened by a craniotomy or catheter puncture; 2) the origin of the bleeding is in the basal subarachnoid space, similar to aneurysmal SAH; and 3) by perforation of a blood vessel, components of the endothelial and muscular layers are exposed to the CSF or are washed into the subarachnoid space. These components may contribute to vascular reactions or coagulation processes. Schwartz and associates24 further examined this experimental model and showed that varying the filament size provides a method to modulate the severity of SAH.

**Acute Vasoconstriction**

Induction of SAH with all filament sizes used created marked elevation of ICP until the end of the observation period. Because of a secondary increase of MABP,
Acute vasoconstriction after experimental SAH

however, only a minor decrease of CPP was observed. In contrast, rCBF was markedly reduced in all SAH groups compared with control animals. This finding most likely resembles acute vasoconstriction starting immediately after induction of SAH. Bilateral parallel occurrence suggests a global vascular reaction and excludes a local vasospastic reaction to the mere insertion of the filament.

Theoretically, a persistent “no-reflow phenomenon” in small arterial or venous blood vessels after the initial ICP spike or an artifactual laser Doppler measurement due to blood in the subarachnoid space could be responsible for these findings. Laser Doppler flowmetry probes

Fig. 1. Graphs showing the time course of ICP (A), MABP (B), and CPP (C). Secondary to a rapid elevation of ICP, MABP rose in the 3-0 and 4-0 groups. Thus, CPP was significantly elevated only 1 minute after SAH. *p < 0.05 versus the control group; §p < 0.05 versus the 4-0 and 5-0 groups.

Fig. 2. Graphs of regional CBF measured by continuous LDF over both hemispheres. A disparity between the minor reduction of CPP (Fig. 1) and the marked and significant reduction of rCBF was found in all 3 SAH groups, indicating acute vasoconstriction, even with smaller filament sizes. Differences between the SAH groups were not significant. The disparity between the CPP and rCBF reduction started at the onset of SAH and persisted until the end of the observation period. *p < 0.05 versus the control group.
were placed on the translucent dura mater and included the subarachnoid space and cortical and subcortical layers to a depth of ~ 1.5 mm. In this space, a mean flow of red blood cells is calculated. If a no-reflow phenomenon was responsible for a persistently reduced rCBF of 50–60% of baseline 2 hours after SAH, morphological changes in the form of cortical lacunar infarctions or microinfarctions should be expected. The microscopic analysis did not show this kind of pathology, nor did we observe territorial infarctions. An artifactual LDF measurement appears to be even less likely because we did not find major blood clots in the subarachnoid space over the parietal convexity. In our experiments, subarachnoid blood was almost exclusively distributed in the basal subarachnoid space. Most likely, traces of blood were spilled into the subarachnoid space over the convexity at the time of vessel injury that were not visible in the postmortem microscopic examination. But—regarding the principles of LDF measurements—minor traces of blood are not likely to cause an artifactual depression of laser Doppler flow to the extent we observed in this study. Our findings instead indicate that there is a uniform vascular reaction after SAH that is largely independent of the decrease of CPP or the extent of hemorrhage.

We did not examine the effect of simply lowering CPP, for example by adding an additional control group in which saline was injected into the subarachnoid space, because we aimed to perform a randomized study and data interpretation could be arguable using different experimental models. However, comparisons of saline injections and blood injections into the subarachnoid space had been previously performed. Ebel et al. found that the injection of saline resulted in a sharp increase of ICP and a parallel increase of MABP, but no marked change of CPP and rCBF, whereas the injection of blood was also followed by a marked decrease of CPP and rCBF. In this regard, those results are in accordance with our observations.

The action of blood products might account for the vasoconstriction observed in our study. However, we found no correlation between the extent of hemorrhage and the reduction of rCBF over both hemispheres. Thus, either a minute volume of blood is sufficient to initiate a uniform vasoconstriction or the injury to the vessel wall itself may be crucial for the development of acute global vasoconstriction, for example by the release of vasoactive endothelial products into the subarachnoid space. It should be noted that a marked discrepancy between CPP and CBF is usually not found in experimental studies using intracisternal blood infusion for induction of SAH, whereas in endovascular puncture models it appears to occur rather consistently. A direct comparison of cisternal infusion models and the endovascular puncture model confirmed this discrepancy, and signs of acute vasoconstriction have also been found in the early stage after aneurysmal SAH in patients. Therefore, the contribution of vascular damage to the compromised perfusion appears to be more likely than the mere presence of subarachnoid blood.

At first glance, these findings appear not to be in accordance with the observations in clinical practice. On hospital admission, some of the patients suffering from SAH are awake, completely aware of the situation, and without neurological deficit. However, CBF has to decrease to values below 50% of normal until neuronal function reversibly ceases and even further to fall below ischemic thresholds. Initial patient unconsciousness or disorienta-
Acute vasoconstriction after experimental SAH

release of Ca\textsuperscript{2+} from intracellular stores or entry of Ca\textsuperscript{2+} through Ca channels in the cell membrane may be the cellular basis for this phenomenon.\textsuperscript{1} To cause periodic contraction of blood vessels, however, oscillatory Ca fluctuations of single cells need to be coordinated. Synchronization might be organized by gap junctions between the smooth muscle cells, and sympathetic fibers may also play a role.\textsuperscript{1,20} Synchronized vasomotion has been observed under pathological conditions including chronic hypertension,\textsuperscript{19} acute malperfusion,\textsuperscript{20} and hemorrhage.\textsuperscript{12}

Little is known about the importance of a vasomotion-like perfusion pattern under physiological or pathophysiological conditions. It has been hypothesized that vasomotion may enhance blood flow and O\textsubscript{2} delivery in states of reduced supply.\textsuperscript{1,20} Whether the oscillatory vascular reaction constitutes a sign of “rescue perfusion” or resembles a mild, initial form of early vasospasm is unknown. Our observation was that the oscillatory vascular reaction occurred more frequently in the groups in which smaller filaments were used and where SAH was less pronounced. Thus, this finding rather supports the possibility that the oscillatory vascular reaction may resemble an initial form of vasospasm. In more pronounced SAH the same processes may occur, but in a more distinct way, shifting to a tonic form of vasoconstriction. If this were the case, inhibiting these processes might have therapeutic potential.

Conclusions

The present results suggest that subjects of all clinical grades, even with only minor hemorrhage, express vasoconstriction after SAH. Even if CBF does not fall below ischemic thresholds, this phenomenon may constitute a reduced basis of CBF. Perfusion and tissue supply may be further endangered by factors such as occlusive hydrocephalus, hypoventilation, or hypotension. The sum of these factors may result in focal or global perfusion deficits. To gain further insight into the pathophysiology of the acute phase after SAH, additional studies are needed to characterize parameters of tissue metabolism and to visualize cerebral vessels in this particular period.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

References


Fig. 4. Graphs showing the correlation between the amount of subarachnoid blood (SAH score) and rCBF after 5 minutes (upper) and after 120 minutes (lower), calculated using the Spearman rank correlation coefficient. Correlation coefficients were very weak (−0.025 and −0.20, respectively) and probability values were not significant (0.90 and 0.34, respectively), demonstrating that rCBF immediately after induction of SAH and after 120 minutes is largely independent of the amount of subarachnoid blood.


Manuscript submitted June 4, 2008. 
Accepted August 8, 2008. 
Please include this information when citing this paper: published online December 5, 2008; DOI: 10.3171/2008.8.JNS08591.

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