An in vitro comparative study of conducting vessels and penetrating arterioles after experimental subarachnoid hemorrhage in the rabbit

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The reactivity of rabbit basilar artery and penetrating arteriolar microvessels was studied in vitro using an isometric-tension measurement technique and an isolated perfused arteriole preparation, respectively. Comparisons were made between reactivities of normal vessels and those obtained from animals subjected to experimental subarachnoid hemorrhage (SAH) 3 days prior to examination. Subarachnoid hemorrhage produced significant increases in basilar artery contraction in response to increasing concentrations of serotonin (5-hydroxytryptamine) \( (10^{-5} \text{ to } 10^{-3} \text{ M}) \) and prostaglandin \( F_2 \), \( (10^{-5} \text{ to } 10^{-3} \text{ M}) \) when compared to normal arteries. In addition, SAH attenuated the relaxing effect of acetylcholine following serotonin-induced contraction and of adenosine triphosphate after KCl-induced basilar artery contractions. In contrast to the changes observed in large arteries, cerebral microvessels did not demonstrate significant differences in spontaneous tone or in reactivity to a number of vasoactive stimuli including application of calcium, serotonin, and acetylcholine. On the other hand, small but significant changes in arteriolar responsiveness to changes in extraluminal pH and to application of KCl were noted.

Findings from this study suggest that intracerebral resistance vessels of the cerebral microcirculation are not greatly affected by the presence of subarachnoid clot, in contrast to the large arteries in the basal subarachnoid space. The small changes that do occur are qualitatively different from those observed for large arteries. These findings are consistent with the observation of significant therapeutic benefit with the use of calcium channel blockers without changes in angiographically visible vasospasm in large vessels. It is likely, therefore, that calcium antagonists may act to decrease total cerebrovascular resistance at the level of the relatively unaffected microcirculation after SAH without changing large vessel diameter.

**Key Words** - microcirculation - cerebral artery - arteriole - vasospasm - subarachnoid hemorrhage - rabbit

Cerebral vasospasm after subarachnoid hemorrhage (SAH) remains a significant cause of death and morbidity despite recent improvements in therapy of this disorder. There is a clear association between the development of delayed cerebral ischemia and the presence of thick subarachnoid clot, which eventually produces sustained constriction of conducting arteries within the subarachnoid space. However, the inconstant relationship between manifestations of cerebral ischemia and angiographically determined vasospasm suggests that the microcirculation may still be capable of vasodilation and preservation of relatively normal cerebral blood flow in some patients with vasospastic narrowing of the more proximal conducting arteries.

Grubb, et al., \(^{11}\) initially demonstrated that cerebral blood volume (CBV) was increased in patients with symptomatic SAH. In the present paper, we have extended these observations to a direct assessment of the pathophysiology of conducting vessels and microvessels removed from rabbits undergoing experimental SAH. The goal of these studies was to determine the extent to which microcirculation is affected by SAH in comparison with more proximal conducting artery segments.

**Materials and Methods**

Male New Zealand White rabbits, each weighing 2.9 to 4 kg, were used for this study. Animal experimentation was conducted in conformity with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication No. 86-23).
Subarachnoid Hemorrhage Model

Rabbits were anesthetized with intramuscular injections of ketamine (30 mg/kg) and xylazine (5 mg/kg), and were endotracheally intubated, paralyzed with pancuronium bromide (0.1 mg/kg), and ventilated with a respirator.* The central ear artery was cannulated, and 4.5 ml of autologous arterial blood was obtained. With aseptic techniques, a sterile No. 23 butterfly needle was placed percutaneously in the cisterna magna. A small amount (0.5 to 1 ml) of cerebrospinal fluid was aspirated and 4.5 ml of autologous nonheparinized blood was injected over a 1- to 2-minute period. The rabbits were then placed in a 45° head-down tilt for 15 minutes to facilitate settling of the blood in the basal subarachnoid cisterns. They were then allowed to recover from anesthesia, were extubated while awake, and were returned to their cages.

The day of SAH is designated Day 0. Previous studies with a rabbit model have determined that maximum vasospasm occurs on post-hemorrhage Days 3 to 4.* We therefore selected Day 3 as the time to examine changes in vascular reactivity associated with SAH. On Day 3, the rabbits were reanesthetized with an intramuscular injection of ketamine and xylazine as noted above, and were sacrificed by exsanguination from the femoral artery. The brain with the basilar artery in situ was removed and placed in a dissecting chamber filled with a modified Krebs bicarbonate solution with a composition of (in mM): NaCl 120, KCl 4.5, MgSO4 1.0, NaHCO3 27.0, KH2PO4 1.0, CaCl2 2.5, and dextrose 10.0.

Basilar Artery Reactivity

For the studies of large-artery reactivity, the basilar artery was dissected free under magnification and divided into 3-mm segments. The ring segments were suspended between two L-shaped stainless-steel rods in an organ bath with a 10-ml working volume of Krebs solution, which was bubbled with 95% O2/5% CO2. The pH of the bathing solution ranged from 7.4 to 7.5. One rod was connected to a force-displacement transducer.† The preparations were allowed to equilibrate at 37°C for 60 minutes prior to use. Preliminary length-tension experiments on basilar artery rings from normal and SAH-treated rabbits were performed to determine the optimum resting length for maximum active force development which corresponded to an average resting tension level of 400 mg; these experiments were therefore performed at that resting tension. Contractile force was recorded isometrically using a force-displacement transducer and was displayed on a polygraph.‡

To confirm sufficient contractile activity in each specimen, the response to 40 mM KCl was first obtained in each ring segment, and only specimens that showed a good response were used for subsequent experiments. There were no significant differences between the control and SAH groups in the number of arteries rejected.

The response of basilar artery segments from normal and SAH-treated rabbits to increasing concentrations of KCl (10, 40, and 80 mM), prostaglandin F2α (PGF2α), and serotonin (5-hydroxytryptamine) (10−6, 10−8, 10−10, 10−12, and 10−14 M) was examined. The relaxing effect of acetylcholine on vessels precontracted with 40 mM KCl or serotonin (10−10 M) was also studied, as was adenosine triphosphate (ATP)-induced relaxation of KCl-induced contractions. For studies of ATP-induced relaxation, 1 mM 8-phenyltheophylline was included in the bath medium prior to exposure to ATP so as to block any vasodilator effects of adenosine that could potentially contaminate the ATP solutions.

Microvessel Reactivity

In a series of parallel experiments, brain-stem perforating arterioles from basilar arteries were isolated and cannulated in an organ bath, and changes in vessel diameter in response to the extraluminal administration of agents were measured. Methods for obtaining perforating arterioles from basilar arteries were modified slightly from the previous description for rat intracerebral arterioles.b,† Briefly, a slab of brain, 10 × 5 × 2 mm thick, with its overlying pia and basilar artery intact was removed from the ventral portion of the midbrain and upper pons. The pial membrane was then reflected gently from the surface of this brain-stem segment with sharpened No. 5 Dumont jeweler’s forceps. The perforating arterioles (30 to 70 μm in diameter) are separated from brain-stem parenchyma by the Virchow-Robin space, thus permitting the vessels to be removed easily from the surrounding parenchyma. The vessel segments were transferred to a temperature-controlled chamber on the stage of an inverted microscope and were cannulated using glass pipettes. Vessel diameter was determined by means of a video dimensional analysis system.

After vessel cannulation, transmural pressure was set at 60 mm Hg via the cannulating pipette, and the bath temperature was brought to 37° or 38°C; the extraluminal bath solution had a pH of 7.3. The vessels developed spontaneous tone during the equilibration period of 45 minutes, contracting to between 60% and 70% of maximum passive diameter at room temperature. Responsiveness of the vessels was then assessed by changing the extraluminal pH from 7.3 to 6.8 and from 7.3 to 7.6. Vessels that showed weak responses (< 15% dilation at a pH of 6.8 or < 15% contraction at a pH of 7.6) were discarded at this stage.

Vessel diameter attained during the equilibration period remained reasonably constant throughout the experiments. Control diameter was defined as the diameter at a bath solution pH of 7.3 immediately before

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* Respirator, Model 683, manufactured by Harvard Instrument Co., South Natick, Massachusetts.
† Force-displacement transducer, Model FT-03, manufactured by Grass Instrument Co., Quincy, Massachusetts.
‡ Polygraph, Model 3200S, manufactured by Gould, Inc., Cleveland, Ohio.
Vessel reactivity after experimental SAH

**FIG. 1.** Graphs showing the effects of exposure to increasing concentrations of serotonin (5-hydroxytryptamine (5-HT), left) and prostaglandin F₄₀ (PGF₂₀, right) on tension development in basilar artery ring segments of control and subarachnoid hemorrhage (SAH)-treated rabbits. Tension is expressed as a percentage of maximum tension development induced by 40 mM KCl in the study of 5-HT and in grams in the study with PGF₂₀. n = number of rabbits in each group. Values are the mean ± standard error of the mean; statistical significance: * = p < 0.05; ** = p < 0.01; + = not significant.

**FIG. 2.** Graphs showing the relaxation of basilar artery ring segments in response to increasing doses of acetylcholine (ACh) in segments precontracted with 40 mM KCl (left) and with 10⁻⁵ M serotonin (right) of control and subarachnoid hemorrhage (SAH)-treated rabbits. n = number of rabbits in each group. Values are the mean ± standard error of the mean; statistical significance: * = p < 0.05 and ** = p < 0.01.

Statistical comparison between the control and SAH groups was made using Student’s t-test for unpaired observations. Values were considered to be significantly different when p < 0.05.

**Results**

**Basilar Artery Reactivity**

Cumulative increases of KCl concentration from control (4.5 mM) to 10, 20, 40, and 80 mM resulted in a maximum increase in tension development between 40 and 80 mM. No significant differences were noted between SAH and normal vessels.

The response of basilar artery ring segments to increasing concentrations of serotonin (10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻⁹ M) was examined and recorded as the percentage of maximum tension induced by 40 mM KCl. Arteries from SAH-treated rabbits showed greater degrees of vasoconstriction to all concentrations of serotonin examined. Statistically significant differences in tone were seen at 10⁻⁷, 10⁻⁶, and 10⁻⁵ M concentrations of serotonin (Fig. 1 left). Calculated EC₅₀ was 6.03 × 10⁻⁸ for SAH vessels and 1.23 × 10⁻⁷ for normal arteries (negative log EC₅₀: 7.22 ± 0.14 and 6.91 ± 0.15, respectively). These latter differences, however, were not significant (p = 0.09).

The response of basilar artery ring segments to the application of PGF₂₀, was also enhanced following SAH (Fig. 1 right). Vessels were exposed to PGF₂₀ concentrations of 10⁻³, 10⁻⁶, 10⁻⁷, 10⁻⁸, and 10⁻⁹ M. Tension development was significantly greater for SAH arteries exposed to concentrations of 10⁻³, 10⁻⁶, and 10⁻⁹ M.

Vessels precontracted with 40 mM KCl were exposed to increasing concentrations of acetylcholine (10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, and 10⁻⁷ M). No significant difference in the relaxation observed with acetylcholine was noted between SAH and normal vessels (Fig. 2 left). A slight vasoconstriction was recorded with low doses of acetylcholine applied to SAH arteries. Significant relaxation was observed at concentrations of 10⁻⁴ and 10⁻³ M. There was a trend toward greater relaxation in normal
vessels; however, this never achieved statistical significance. The relaxing effect of acetylcholine was also examined for serotonin-induced contractions in both normal and SAH arteries (Fig. 2 right). In this case, normal arteries relaxed significantly more to doses of 10^{-7}, 10^{-6}, and 10^{-5} M acetylcholine.

Arteries precontracted with 40 mM KCl were also exposed to increasing doses of ATP in the presence of 8-phenyltheophylline. At doses greater than 10^{-7} M, ATP-induced relaxation was observed; this was significantly greater in normal arteries when compared to arteries examined following SAH (Fig. 3).

**Microvessel Reactivity**

The passive arteriolar diameter, as determined for each vessel at the beginning of the experimental protocol in an extraluminal bath solution of pH 7.3 at 60 mm Hg transmural pressure and 23°C, averaged a mean of 83.8 ± 4.8 μm for 10 normal rabbits and 79.3 ± 4.1 μm for nine SAH-treated rabbits. After the development of spontaneous tone at 37°C, control vessel diameter averaged 49.0 ± 3.1 μm for normal rabbits and 47.5 ± 3.3 μm for SAH-treated animals. These values were not significantly different from each other (p = 0.75).

When the pH of the extraluminal solution was lowered from 7.3 to 6.8, arterioles dilated to a similar extent (p = 0.12) between 10 normal rabbits (143.0% ± 2.6% of control vessel diameter) and nine SAH-treated rabbits (149.0% ± 2.5%). On the other hand, when the pH was raised to 7.6, arterioles from nine SAH-treated rabbits constricted to a significantly greater degree (69.3% ± 0.9% of control vessel diameter) than those from 10 normal rabbits (76.2% ± 1.5% of control vessel diameter; p = 0.00045 × 10^{-4} M).

When the extraluminal solution was changed from PSS to Ca^{++}-free solution, arterioles dilated to 152.4% ± 2.3% of control vessel diameter in five normal rabbits and to 163.3% ± 6.7% in five SAH-treated rabbits (Fig. 4). These values were not significantly different from each other (p = 0.17). Vessel diameters gradually returned to the control diameter when the concentration of Ca^{++} was increased to 2.5 mM, which is the calcium concentration of PSS. The EC_{50} values were 1.66 mM (negative log EC_{50}: 2.78 ± 0.12) for normal rabbit arterioles and 0.76 mM (negative log EC_{50}: 3.12 ± 0.03) for the SAH group arterioles; these values were also not significantly different from each other (p = 0.12).

Extraluminal KCl concentrations were increased cumulatively from control (3.0 mM) to 10, 20, 40, and 80 mM. Potassium chloride induced a biphasic response in penetrating arterioles as concentrations increased; lower concentrations such as 10 and 20 mM produced vasodilation and high concentrations such as 40 and 80 mM produced vasoconstriction (Fig. 5).

Arterioles dilated markedly at 10 mM KCl to 149.7% ± 1.1% of control vessel diameter in five normal rabbits and to 163.2% ± 3.5% in five SAH-treated rabbits. These values were not significantly different from each other (p = 0.052). At a KCl concentration of 20 mM, arterioles slightly decreased in diameter; however, arteriolar diameters remained greater than baseline in both groups (143.9% ± 1.6% in normal rabbits and 158.9% ± 2.0% in SAH-treated rabbits). These values were significantly different from each other (p = 0.0061), suggesting that arterioles from the SAH-treated rabbits dilated more than those from normal rabbits at 20 mM KCl. When KCl concentrations were increased to 40 mM, arterioles changed from a vasodilated to a vasoconstricted state with vessel diameters of 74.0% ± 3.1% in normal rabbits and 58.5% ± 1.7% in the SAH group. Arterioles from SAH-treated rabbits demonstrated significantly greater vasoconstriction than those from normal rabbits at 40 mM KCl (p = 0.023). When KCl concentrations were increased further to 80 mM, arterioles constricted a little more to 61.4% ± 3.8% in normal rabbits and 52.3% ± 1.1% in the SAH-treated rabbits. These values were not significantly different from each other (p = 0.184).

Serotonin produced vasoconstriction in a dose-dependent manner with maximum vasoconstriction to 91.1% ± 1.5% of control vessel diameter between 10^{-7} to 10^{-5} M.
Vessel reactivity after experimental SAH

![Graph showing the response of perforating arterioles in control and subarachnoid hemorrhage (SAH)-treated rabbits](image)

**FIG. 5.** Graph showing the response of perforating arterioles in control and subarachnoid hemorrhage (SAH)-treated rabbits to various concentrations of extraluminal KCl. n = number of rabbits in each group. Values are the mean ± standard error of the mean; statistical significance: p < 0.05.

and $10^{-5}$ M in five normal rabbits and $90.4\% \pm 1.5\%$ between $10^{-6}$ and $10^{-5}$ M in four SAH-treated rabbits (Fig. 6 left). These values are not significantly different from each other ($p = 0.754$). The EC_{50} values were $4.37 \times 10^{-7}$ M (negative log EC_{50}: 7.36 ± 0.11) for normal rabbit arterioles and $1.26 \times 10^{-7}$ M (negative log EC_{50}: 6.90 ± 0.11) for SAH-treated rabbit arterioles; these values are also not significantly different from each other ($p = 0.076$).

Acetylcholine induced vasodilation in a dose-dependent manner in rabbit basilar perforating arterioles, with maximum dilation to 152.8% ± 3.7% of control vessel diameter in five normal rabbits and 159.9% ± 5.0% in five SAH-treated rabbits (Fig. 6 right). These values were not significantly different from each other ($p = 0.29$). The EC_{50} values were $1.26 \times 10^{-7}$ M (negative log EC_{50}: 6.90 ± 0.09) for normal rabbit arterioles and $1.15 \times 10^{-7}$ M (negative log EC_{50}: 6.94 ± 0.04) for the SAH group arterioles, without significant difference from each other ($p = 0.80$).

**Discussion**

There is often a lack of close correlation between the symptoms of delayed cerebral ischemia after SAH and angiographic vasospasm.2,12,20 This apparent paradox has led some investigators to hypothesize that the cerebral microcirculation is affected by cerebral vasospasm.27

**Intracerebral Vasodilation in Cerebral Vasospasm**

In a series of classic observations, Grubb and colleagues.21 demonstrated that cerebral perfusion and oxygen utilization were decreased in 30 patients who had suffered an SAH. These investigators observed a nearly 60% increase in CBV in patients with large-vessel spasm on cerebral arteriography. They concluded from these studies that vasospasm of large subarachnoid arteries was accompanied by marked dilation of the parenchymal vessels.22 In a follow-up study using positron emission tomography,23 they confirmed the observation that patients with vasospasm have reduction in cerebral blood flow associated with marked increases in CBV, implying an autoregulatory response of the parenchymal resistance vessels to decreased perfusion pressure.

**Heterogeneity of Cerebrovascular Responsiveness**

The physiological responsiveness of intracerebral microvessels is clearly different in many cases from that of large conducting cerebral vessels.4,7 Cerebral microvessels appear to be particularly sensitive to changes in extracellular calcium concentration. Auer, et al.,7 observed that nimodipine infused intravenously in patients undergoing extracranial-intracranial bypass procedures dilated smaller pial vessels to a greater degree than large subarachnoid arteries. They also postulated a similar increase in the sensitivity of the parenchymal arteriolar bed. Takayasu, et al.,23 studied the responsiveness of isolated penetrating intracerebral arterioles to calcium channel blockers and found them to be more sensitive to these agents than larger, angiographically visible arteries.

These differences in reactivity between arterioles and larger conducting vessels may explain the discrepancy between the clinical effectiveness of agents such as nimodipine in preventing ischemic symptoms and the lack of observed effects on angiographic spasm.1,10,19,20 It is important to remember, however, that the studies of Auer and coworkers11 and Takayasu, et al.,23 were performed on vessels from normal animals. The reactivity of arteriolar vessels after SAH might be significantly different.

**Effects of SAH on Intraparenchymal Resistance Vessels**

The goal of the present study was to determine whether arteriolar microvessels immediately subjacent to a subarachnoid clot demonstrate significant changes...
in their reactivity, analogous to what has been frequently observed in vitro in large conducting arteries. In this series of experiments, we were able to demonstrate in vitro many of the changes in reactivity previously observed for large subarachnoid arteries.\textsuperscript{18,21,24,25} Significant increases in serotonin reactivity and reactivity to PGF\textsubscript{2\alpha} were noted in basilar artery segments. In addition, SAH was found to attenuate the vasodilation observed in response to acetylcholine and ATP. In contrast, however, SAH had little effect on the reactivity of the penetrating arterioles. Specifically, the responses to serotonin and acetylcholine were not altered 3 days following the SAH, a time when the immediately contiguous basilar artery reactivity was significantly changed.

We noted a minor alteration in arteriolar reactivity to extraluminally applied KCl. Subarachnoid hemorrhage significantly decreased the relaxation observed at 20 mM KCl and attenuated the vasoconstriction noted at 40 mM KCl. The basis for these differences is not clear, although it is likely that changes in the resting membrane potential or potassium conductance, as suggested by Harder, et al.,\textsuperscript{13} could be expected to alter the response to 20 mM and 40 mM KCl. Arterioles showed a somewhat enhanced reactivity to elevated pH. Impaired contractility was not observed. In contrast to the observations in small (150 to 200 \textmu m) pial arterioles by Bevan, et al.,\textsuperscript{7} we did not observe changes in spontaneous tone in cerebral microvessels after SAH.

The present study indicates that the reactivity of the intraparenchymal resistance vessels is not significantly altered after SAH. The most likely reason for this finding is the failure of subarachnoid clot to extend along the Virchow-Robin spaces surrounding the penetrating arterioles. Hutchings and Weller\textsuperscript{13} studied the human pial anatomy in this regard and found that a pial barrier exists between the subarachnoid and perivascular spaces that excludes particulate material such as erythrocytes from the latter. Scanning electron microscopy in the rabbit supports such a concept for this species.\textsuperscript{15} This barrier might serve to minimize any direct effects of subarachnoid blood at the arteriolar level.

The results of the present study obtained in a rabbit model are consistent with the observations of Grubb and colleagues,\textsuperscript{11,16} suggesting that the cerebral microcirculation may in fact become vasodilated in a setting of severe cerebral vasospasm. It is likely that as vaso- spas tic narrowing of large conducting cerebral arteries progresses, the cerebral microcirculation vasodilates to compensate for an increase in overall cerebrovascular resistance. At some point, however, the ability of the intracerebral resistance vessels to compensate is exceeded and cerebral ischemia occurs. This ability of the intracerebral microcirculation to compensate for changes in vascular resistance within the conducting vessel segments may partly explain the commonly observed discrepancy between the degree of angiographically demonstrated vasospasm and symptoms of cerebral ischemia.
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