Impairment of Endothelium-Dependent Relaxation in Human Basilar Artery After Subarachnoid Hemorrhage

Katsuhiko Hatake, MD, PhD; Ichiro Wakabayashi, MD, PhD; Eizo Kakishita, MD, PhD; and Shigeru Hishida, MD, PhD

Background and Purpose: The goal of this study was to determine the alterations in vascular reactivity of human basilar artery after subarachnoid hemorrhage.

Methods: Human basilar arteries were obtained from subjects who died within 1 day after subarachnoid hemorrhage and control subjects who died from causes other than brain involvement. Basilar artery strips were suspended for isometric tension recording in Krebs-Ringer solution. Morphometric study was also carried out on paraffin-embedded sections stained with van Gieson's elastica stain of preselected sites from the basilar arteries. The intimal and medial area and the intimal index ([intimal area/area circumscribed by internal elastic lamina] x 100) were evaluated.

Results: Contractile responses to KCl, norepinephrine, and 5-hydroxytryptamine did not differ between subarachnoid hemorrhage and control groups. The endothelium-dependent relaxation responses to thrombin, bradykinin, and calcium ionophore A23187 were less for the subarachnoid hemorrhage group than for the control group. However, the endothelium-independent response to sodium nitroprusside of the subarachnoid hemorrhage group did not differ from that of the control group. Morphometric measurements were comparable between the two groups.

Conclusions: These results suggest that the decreased relaxation responses to thrombin and bradykinin occur at the level of endothelial cells and not smooth muscle cells and that decreased relaxation may be involved in delayed vasospasm after subarachnoid hemorrhage. Although the decreased relaxation was observed within 1 day after subarachnoid hemorrhage, a period in which delayed spasm does not occur, this time difference may be dependent on the severity of bleeding after rupture of an aneurysm. (Stroke 1992;23:1111–1117)

KEY WORDS • basilar artery • endothelium • subarachnoid hemorrhage

Cerebral arterial vasospasm is a major cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH) after rupture of an intracranial aneurysm. Despite intensive research, the basic pathogenesis of the vasospasm remains unclear. Several substances in the cerebrospinal fluid have been implicated in its etiology: catecholamines,1 5-hydroxytryptamine,2 prostaglandins,3 4 fibrin–fibrinogen degradation products,5 oxyhemoglobin,6 7 and neuropeptide Y.8 Furthermore, an imbalance in prostanoyl and thromboxane A2 production in the cerebral arterial wall,9 10 the potent vasoconstrictor effect of endothelin, which is released by the vascular endothelium,11 12 increased contraction due to denervation supersensitivity13 14 or the development of proliferative vasculopathy15 may be involved in the pathogenesis of cerebral vasospasm. Thus, the pathogenesis of the delayed spasm appears to be multifactorial.

An intact vascular endothelium has been shown to play an obligatory role in producing relaxation via endothelium-derived relaxing factor (EDRF) in response to a number of agonists.16 17 Recently, decreased endothelium-dependent relaxation in response to several agonists after SAH has been reported to be involved in delayed vasospasm after subarachnoid hemorrhage. Although the decreased relaxation was observed within 1 day after subarachnoid hemorrhage, a period in which delayed spasm does not occur, this time difference may be dependent on the severity of bleeding after rupture of an aneurysm. (Stroke 1992;23:1111–1117)

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Materials and Methods

Basilar arteries were carefully removed from subjects who died within 1 day after SAH (SAH group) and...
Table 1. Source of Human Basilar Arteries Used in Contraction and Relaxation Experiments

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yr)/sex</th>
<th>Site of aneurysm</th>
<th>Cause of death</th>
<th>Case No.</th>
<th>Age (yr)/sex</th>
<th>Site of aneurysm</th>
<th>Cause of death</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>56/F</td>
<td>AMI</td>
<td></td>
<td>12</td>
<td>50/M</td>
<td></td>
<td>Asphyxia</td>
</tr>
<tr>
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<td>Bronchial asthma</td>
<td></td>
<td>13</td>
<td>66/M</td>
<td></td>
<td>Asphyxia</td>
</tr>
<tr>
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<td>59/M</td>
<td>Hemorrhage</td>
<td></td>
<td>14</td>
<td>57/M</td>
<td></td>
<td>AMI</td>
</tr>
<tr>
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<td>48/M</td>
<td>TBC</td>
<td></td>
<td>15</td>
<td>74/F</td>
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<td>Gastric cancer</td>
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<tr>
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<td>43/M</td>
<td>Liver cirrhosis</td>
<td></td>
<td>16</td>
<td>49/M</td>
<td></td>
<td>APN</td>
</tr>
<tr>
<td>6</td>
<td>53/M</td>
<td>AMI</td>
<td></td>
<td>17</td>
<td>46/F</td>
<td></td>
<td>Liver cirrhosis</td>
</tr>
<tr>
<td>7</td>
<td>57/M</td>
<td>Right ACA</td>
<td></td>
<td>19</td>
<td>63/F</td>
<td></td>
<td>Right MCA</td>
</tr>
<tr>
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<td>Left MCA</td>
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<td>20</td>
<td>49/F</td>
<td></td>
<td>Right ACA</td>
</tr>
<tr>
<td>9</td>
<td>59/M</td>
<td>ACOM</td>
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<td>53/F</td>
<td></td>
<td>Right MCA</td>
</tr>
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<td>Left ACA</td>
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| 15 minutes, and the hooks were then adjusted to give a resting tension of 1.5 g. The contraction experiment was conducted with samples from case No. 1 to No. 6 of the control group and No. 7 to No. 11 of the SAH group (Table 1). The endothelium was removed from one strip each of the six cases of the control group by rubbing the intimal surface with filter paper.16 Successful removal of endothelial cells from aortic strips was confirmed by the inability of 10^{-5} M bradykinin, an endothelium-dependent vasodilator,23 to induce relaxation, and by histological examination of the intimal surface, using a silver staining technique.24 In both groups, KCl (10^{-6}-10^{-3} M), and 5-hydroxytryptamine (10^{-6}-10^{-3} M) were cumulatively added to the organ bath. Contraction by KCl was expressed as milligrams tension/milligrams tissue weight and that by norepinephrine and 5-hydroxytryptamine was expressed as a percentage of the contraction elicited by 60 mM KCl. The relaxation experiment was conducted for case No. 12 to No. 18 of the control group and No. 19 to No. 25 of the SAH group (Table 1). Strips from both groups were subjected to precontraction with 10^{-6} M phenylephrine, and then bradykinin (10^{-6}-10^{-3} M), calcium ionophore A23187 (10^{-8}-10^{-6} M), and sodium nitroprusside (10^{-9}-10^{-6} M) were cumulatively added to the organ bath. However, for the thrombin experiment, application of 5 National Institutes of Health (NIH) units/ml was done only once to each strip, because the relaxation by thrombin resulted in tachyphylaxis toward a second exposure, which prevented data collection for a dose-response curve.23

In a separate experiment, one strip from each subject in the SAH group was exposed to 10^{-5} M indomethacin for 60 minutes before the addition of phenylephrine. The vascular responses to thrombin and bradykinin in the presence of indomethacin were compared with...
those induced by each agonist in its absence using another strip from the same subject. Relaxation was expressed as a percentage of contraction in response to $10^{-6}$ M phenylephrine. The drugs were added to the organ bath medium in a volume of 50–100 µl, and their final concentrations in the medium are given in the text.

Morphometric study was carried out on a paraffin-embedded section using the other segment from the basilar artery. Cross sections were cut at approximately 1-mm intervals to obtain five arterial sections. The sections were stained with van Gieson's elastica stain, and morphometric determination was performed with an image analyzer system (Quantimet 720, Cambridge Instrument, Cambridge, England) to evaluate the area of the intima and media and the length of the internal elastic lamina, as previously described.25-26 The intimal index, an index of intimal thickening, was calculated by dividing the area of the intima by the area enclosed by the internal elastic lamina (internal elastic lamina area) in its theoretically unwrinkled state, according to the following formula:

\[
\text{intimal index} = \frac{\text{intimal area}}{\text{corrected internal elastic lamina area}} \times 100
\]

The corrected internal elastic lamina area was calculated from the formula \(\frac{\text{length of internal elastic lamina}^2}{4\pi}\), where internal elastic lamina is the length of the lamina in millimeters. Each measurement was performed for the five sections obtained from one segment, and the mean value was calculated to avoid site differences.

Bradykinin was purchased from Peptide Institute Inc., Osaka, Japan. Bovine thrombin (1,280 NIH units/mg) was obtained from Mochida Pharmaceutical Co., Ltd., Tokyo, Japan. 1-Norepinephrine hydrochloride, 1-phenylephrine hydrochloride, 5-hydroxytryptamine, calcium ionophore A23187, and sodium nitroprusside were all obtained from Sigma Chemical Co., St. Louis, Mo.

The data are expressed as mean±SEM. The concentrations needed to reach 50% maximal contraction (ED50 values) and relaxation (ID50 values) were determined graphically after linear regression of the 20–80% region of the log concentration–response curves (norepinephrine, $10^{-6}$–$5 \times 10^{-7}$ M; 5-hydroxytryptamine, $10^{-8}$–$5 \times 10^{-7}$ M; bradykinin, $3 \times 10^{-9}$–$3 \times 10^{-7}$ M). Student's unpaired \(t\) test was used for statistical comparisons of two groups. Comparison of values obtained in the SAH group before and after treatment with indomethacin was performed by the paired \(t\) test. The level of significance was \(p<0.05\).

**Results**

As shown in Table 1, the mean value of age in the SAH group was not different from that in the control group in both contraction and relaxation experiments. Table 2 shows the mean values of intimal and medial area and also the intimal index for the control and SAH groups. In both contraction and relaxation experiments, no significant differences were found in any of these values between the two groups.

Dose-response curves for KCl-, norepinephrine-, and 5-hydroxytryptamine–induced contractions did not show significant differences between the control and SAH groups (Figure 1). The contractile response to norepinephrine or 5-hydroxytryptamine did not show significant differences in vascular strips with and without endothelium in the control group (Table 3). Endothelium-dependent relaxation in response to thrombin (5 units/ml) was significantly less in the SAH group than

![Figure 1](http://stroke.ahajournals.org)  
**Figure 1.** Graphs show dose–response curves for KCl-, norepinephrine-, and 5-hydroxytryptamine–induced contractions in control and subarachnoid hemorrhage (SAH) groups. Each point is mean of six observations in control group and five observations in SAH group. Vertical bars show SEM.
in the control group (Figure 2). Also, the endothelium-dependent relaxations in response to bradykinin and A23187 were significantly less at almost all concentrations tested for the SAH group than for the control group (Figures 2 and 3). However, the dose–response curve for the endothelium-independent relaxation in response to sodium nitroprusside was comparable in the two groups (Figure 3). The relaxation response to 5 units/ml thrombin and the dose–response curve of bradykinin in the SAH group did not significantly differ whether indomethacin was present or absent (the respective data are as follows: for thrombin, 8.1±5.1% and 8.6±4.3%, n=7; for bradykinin, $I_D_{50}$, 9.3±1.9×10^{-9} M; maximal relaxation, 45.7±8.4% and $I_D_{50}$, 8.9±2.5×10^{-9} M; maximal relaxation, 40.9±10.3%, n=7).

Discussion

In the present study, the contractile response to KCl, norepinephrine, and 5-hydroxytryptamine did not show any significant difference between the control and SAH groups. This result suggests that the ability of the artery to produce a contractile response to these agonists in the SAH group, at least functionally, was well preserved. In experimental animal models of SAH, the contractile response has been observed to increase because of denervation supersensitivity caused by the presence of blood clots in the subarachnoid space. In contrast, reduction in the contractile responses due to a functional or structural derangement of contractile elements of smooth muscle cells has also been reported. However, our result, which showed no change in the contractile response, is not necessarily inconsistent with these reports that the changes (i.e., increase or decrease) in contractile response occur after SAH, because the arteries used in the above studies had not been obtained 2–7 days after SAH, whereas in our study they had been obtained within 1 day. Thus, the lapse of time after SAH may be an important factor causing change in the contractile response to vasoactive substances. In a previous study, the cerebrospinal fluids collected from patients with SAH produced greater contractions of normal human basilar arteries than those from patients without SAH because of their higher concentrations of 5-hydroxytryptamine. Thus, although the contraction in response to 5-hydroxytryptamine did not increase following SAH in the present study, we cannot exclude the role of 5-hydroxytryptamine as a spasmogen, because of its higher concentration.

Recently, delayed vasospasm has been thought to be related to an impairment of endothelium-dependent relaxation. In a previous study with human basilar arteries, the endothelium-dependent relaxations induced by thrombin, bradykinin, and calcium ionophore A23187 were inhibited by methylene blue (a guanylate cyclase inhibitor) and bromophenacyl bromide (an inhibitor of EDRF-mediated relaxation) but not by indomethacin (a cyclooxygenase inhibitor). Thus, the three vasodilators share a common susceptibility to these inhibitors, suggesting that these vasodilators can produce relaxation via a similar EDRF-like substance(s). In the present study, endothelium-dependent relaxation in...
response to thrombin and bradykinin was significantly depressed in the SAH group compared with the control group. However, the endothelium-independent relaxation in response to sodium nitroprusside, which, like EDRF, activates soluble guanylate cyclase in smooth muscle cells and produces relaxation, did not differ between the two groups. Also, the increase in the amount of medial smooth muscle after SAH may result in a relative shortage of the EDRF-like substance to cause relaxation, and thereby may lead to the decreased relaxation. However, this is an unlikely explanation because there was no difference in the area of medial smooth muscle between the two groups. Therefore, decreased relaxation probably occurs at the level of the endothelium and not smooth muscle cells.

The decreased relaxation in the SAH group may be due to one or more of the following factors. First, intimal thickening of arterial wall has been observed after SAH. Therefore, disturbances in the transport of the EDRF-like substance to smooth muscle cells and accelerated destruction of this substance may play a role in the decrease of relaxation. However, this possibility is unlikely because the intimal area and intimal index in morphometric analysis of intimal thickening did not show any significant difference between the two groups. A second possibility is that vasoconstrictor substances, such as prostaglandins and endothelin, are involved in delayed vasospasm after SAH. These substances, which may be released together with EDRF in response to agonists, may inhibit endothelium-dependent relaxation. However, as the relaxation response to thrombin and bradykinin in the SAH group was not affected by indomethacin, cyclooxygenase products such as prostaglandin F_2 alpha do not appear to be involved in the decreased relaxation, although the involvement of endothelin cannot be ruled out. A third possibility is that an inflammatory process occurs in the arterial wall after SAH. Inflammatory cells such as macrophages can release oxygen radicals that destroy EDRF. Thus, accelerated destruction of an EDRF-like substance may be a mechanism contributing to impaired vasorelaxation. The most plausible explanation is that loss of the relaxation results from a decrease in the ability of the endothelium to produce and/or liberate an EDRF-like substance. A decrease in relaxation is not likely to occur at the level of receptors on endothelial cells, because the relaxation response to calcium ionophore A23187, which causes endothelium-dependent relaxation in a manner unrelated to any receptor mechanism, led to decreased relaxation in the SAH group. Such ultrastructural changes as the appearance of vacuoles and dense bodies in endothelial cells, which are observed as early as 2 hours after SAH, may be responsible for the decreased production and/or release of an EDRF-like substance.

In the experimental model with rabbit basilar arteries, norepinephrine and 5-hydroxytryptamine produced greater contractions after SAH; the basilar arteries also showed a decrease in endothelium-dependent relaxations. Thus, a decrease in endothelium-dependent relaxations after SAH may raise the possibility that norepinephrine and 5-hydroxytryptamine result in greater contraction in the SAH group than in the control group. However, in our study, these agonists did not produce greater contractions after SAH. The reason for this difference may be as follows. In normal rabbit basilar artery, the contraction in response to norepinephrine and 5-hydroxytryptamine was found to be greater in endothelium-denuded arteries than in endothelium-intact arteries. However, in normal canine and human basilar arteries obtained from the control group, the contraction to them did not differ in the two types of strips. Thus, in contrast to rabbit basilar arteries, vasodilator substance(s) in amounts sufficient to significantly counteract the contractile response may not be released from endothelial cells of normal canine and human basilar arteries. For this reason, even if endothelium is damaged or endothelium-dependent relaxation is impaired, the contraction in response to these agonists may not be potentiated after SAH.

In contrast to the comparable contractile forces in the two groups, endothelium-dependent relaxation was decreased in the SAH group. This result suggests that the endothelium is functionally impaired at an earlier stage than in smooth muscle cells. The decreased relaxation may be responsible for delayed cerebral spasm, as previously reported. However, an important point to note is that decreased endothelium-dependent relaxation in experimental animal models has been observed 2–8 days after SAH, during which the delayed spasm becomes most commonly apparent, but in our study the decreased relaxation was observed in preparations obtained within 1 day after SAH. Therefore, the fact that the decrease in the relaxation response was observed in a period in which delayed spasm does not occur raises the question of whether the decreased relaxation plays a causative role in the spasm. In the present study, the degree of bleeding in the SAH subjects was so severe that they are likely to have died a short time after rupture of the aneurysm. Thus, our SAH cases obtained at medicolegal autopsy may be different from usual clinical treatment cases in which there is survival until delayed spasm occurs. The severity of bleeding may explain the difference in time during which decreased relaxation is observed between experimental animal models and the present study. Therefore, time difference does not necessarily mean that a decrease in endothelium-dependent relaxation is not associated with delayed vasospasm. However, further work is needed to clarify this. Another possibility is that decreased endothelium-dependent relaxation may occur as early as 1 day after SAH even in clinical cases in which delayed spasm is observed. If this is true, how a decrease in the relaxation leads to delayed spasm remains unknown.

In conclusion, the decreased endothelium-dependent relaxations in response to thrombin and bradykinin that are generated during blood coagulation were demonstrated to occur in preparations obtained from subjects who died after SAH, although the decreased response was observed at an earlier stage in our SAH cases than in experimental animal models of SAH.

References

Mechanisms that account for vasospasm after subarachnoid hemorrhage are controversial and may be multifactorial. One mechanism that may contribute to vasospasm is inhibition of endothelium-dependent mechanisms that normally influence cerebral vascular tone. Under basal conditions and in response to specific stimuli, blood vessels release endothelium-derived relaxing factor (EDRF), which inhibits vascular tone by activating guanylate cyclase and increasing cyclic guanosine 5'-monophosphate (GMP) levels in smooth muscle. Inhibition of production or activity of EDRF contracts cerebral arteries under resting conditions and impairs relaxation in response to endothelium-dependent agonists.

Endothelium-dependent relaxation is impaired in experimental models of subarachnoid hemorrhage. The mechanism that produces this impairment may involve decreased production of EDRF or decreased formation of endothelium-derived relaxing factor in rabbit basilar artery. In this issue of Stroke, Hatake et al provide the first evidence that subarachnoid hemorrhage is associated with decreased production of EDRF in canine basilar arteries after subarachnoid hemorrhage.
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