Reduced Production of cGMP Underlies the Loss of Endothelium-Dependent Relaxations in the Canine Basilar Artery After Subarachnoid Hemorrhage

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Endothelium-dependent relaxations are inhibited during chronic vasospasm after subarachnoid hemorrhage in the canine basilar artery, although the luminal release of endothelium-derived relaxing factor (EDRF) is maintained. The present study investigated the mechanisms underlying the impaired vascular reactivity and in particular whether the loss of responsiveness of the smooth muscle to EDRF is due to an impaired production of cGMP. Bradykinin and nitric oxide evoked concentration-dependent relaxations in isolated canine basilar arteries with and without endothelium, respectively, which were reduced in the subarachnoid hemorrhage group. Relaxations evoked by M&B22,948 (an inhibitor of cGMP phosphodiesterases) were smaller, but those evoked by the lipophilic cGMP analogue 8-bromo-cGMP were potentiated slightly in the subarachnoid hemorrhage group. The resting levels of cGMP in rings with endothelium (reflecting the effect of spontaneous release of EDRF) and those evoked by bradykinin in rings with endothelium and by nitric oxide in rings without endothelium were diminished in the subarachnoid hemorrhage group. These data indicate that the altered endothelium-mediated relaxations of the smooth muscle after subarachnoid hemorrhage is due, at least in part, to an impaired activation of soluble guanylate cyclase leading to a reduced production of cGMP in the smooth muscle. (Circulation Research 1992;70:248–256)

The vascular endothelium releases relaxing factor(s) in response to various pharmacological and physiological stimuli.1,2 The best characterized of these factors, endothelium-derived relaxing factor (EDRF), activates soluble guanylate cyclase in vascular smooth muscle and increases cGMP, thus causing relaxation.3–5 Endothelium-dependent relaxations are inhibited by hemoglobin, which scavenges the factor, and also by methylene blue, which inhibits the relaxations by oxidizing soluble guanylate cyclase.6,7 It is likely that EDRF is nitric oxide or a donor of nitric oxide.8–10

In the canine model of chronic vasospasm after subarachnoid hemorrhage, endothelium-dependent relaxations of the isolated canine basilar artery evoked by bradykinin, arginine vasopressin, and thrombin are inhibited.11,12 By contrast, the endothelium-dependent contractions13 evoked by acetylcholine, arachidonic acid, the calcium ionophore A23187, and ADP, as well as by hypoxia and mechanical stretch, are maintained.11 The luminal release of EDRF as determined under bioassay conditions is not reduced after subarachnoid hemorrhage.12 These results suggest that the cause of the loss of endothelium-dependent relaxations is due to reduced transfer of EDRF from endothelial cells to the smooth muscle cells or to an altered responsiveness of the smooth muscle to the factor. The present study was designed to assess the responsiveness of the arterial smooth muscle to EDRF as well as to other cGMP-dependent relaxing agents and to determine whether dysfunction in the production of cGMP underlies the loss of endothelium-dependent relaxations associated with chronic cerebral vasospasm.

Materials and Methods

Animal Model

The double hemorrhage canine model was used.11,12,14–16 Thirty-six mongrel dogs of either sex (weighing 16–23 kg) were allocated randomly to the control and the subarachnoid hemorrhage groups. In

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Supported in part by grants R01 NS-24329-02 and HL-31183 from the National Institutes of Health.

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Received April 24, 1990; accepted October 4, 1991.
the latter, subarachnoid hemorrhage was induced by percutaneous injections of autologous venous blood into the cisterna magna. Under general anesthesia (pentobarbital 15 mg/kg i.v. and thiopental 15–25 mg/kg i.v.) and controlled ventilation, animals were placed in the prone position with the neck flexed 30° down. The cisterna magna was punctured aseptically with a spinal needle (22 gauge, 2.5 in.), and 6–7 ml cerebrospinal fluid was withdrawn and replaced with an equal volume of autologous venous blood. The animals were maintained in the position for 30 minutes with the mechanical ventilation continued for the initial 15 minutes. Two days later, they were anesthetized again and 6–7 ml venous blood was injected in the cisterna magna in the same fashion. An identical procedure yielded reproducible vaso-
spasms of the basilar artery in previous studies. The accumulated angiographical data in these series were as follows: in the subarachnoid hemorrhage group, the cross-sectional area of the basilar artery 7 days later was decreased to 37.0±6.1% (mean±SEM, n=28) of that observed before hemorrhage, whereas in the control group, the ratio was 106.0±6.1% (mean±SEM, n=28). Because of the reliable reproduction of vaso
cspasm, angiography was not performed in the present study. On the eighth day, the animals were killed with an intrave
nous dose of sodium pentobarbital (30 mg/kg) followed by exsanguination. The brain and the cervical cord were dissected free. With the use of a stereomicroscope, the basilar artery was separated from the brainstem and the subarachnoid clot was removed carefully. The artery was cut into rings (about 4 mm long) for the relaxation studies and the measurement of cGMP in the tissue. The care of the animals and procedures in the study complied with the “Prin
ciples of Laboratory Animal Care” and the “Guide for the Care and Use of Laboratory Animals” (DHHS pub
cation No. [NIH] 85-23, revised 1985) and had been approved by the Institutional Animal Care and Use Committee of the Mayo Foundation.

In Vitro Studies

In some preparations, the intima was removed mechanically by gently rubbing the inner surface with a stainless-steel wire. Pharmacological experiments were carried out in a paired fashion on rings from control and subarachnoid hemorrhage animals. Each ring was connected to an isometric force transducer (model UC-2, Gould Inc., Cleveland, Ohio) by means of a pair of triangular stainless-steel wire stirrups (0.035 gauge) inserted in the lumen and suspended in a 37°C organ chamber filled with modified Krebs
-Ringer bicarbonate solution (millimolar composition: NaCl 118.3, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, CaEDTA 0.026, and glucose 11.1; control solution) bubbled with a 95% O2–5% CO2 gas mixture. The rings cut from the basilar artery were irrigated with fresh control solution three times and equilibrated for 30 minutes at a length for which the tension was 100 mg, which is referred to as the initial length. The rings were then placed at the optimal point of their length–tension relation by using potassium chloride (20 mM). The length of stretching required to reach the optimal point from the initial length was significantly shorter in the subarachnoid hemorrhage group than in the control group (rings with endothelium, 0.73±0.02 mm [n=18] versus 0.96±0.03 mm [n=16], p<0.0005; rings without endothelium, 0.79±0.02 mm [n=29] versus 1.03±0.02 mm [n=27], p<0.0005), a finding charac
teristic to the arteries in chronic vasospasm. After each application of potassium chloride, the chamber was washed three times with fresh control solution. The ED50 (3×10–5 M) for UTP, determined in a previous study to be comparable in control and subarachnoid hemorrhage arteries, was used to con
tract the rings before application of the relaxing agents. The following relaxants were tested: bradyki
nin, nitric oxide, 8-bromo-cGMP, and M&B22,948 (a selective cGMP phosphodiesterase inhibitor). In each ring, one cumulative concentration–response curve was obtained to a single relaxing agent. At the end of the concentration–response curve, the maxi
mal relaxations were obtained in each ring with papaverine (3×10–4 M) plus diltiazem (10–4 M). The effects of methylene blue (an inhibitor of soluble guanylate cyclase) and oxyhemoglobin (a scavenger of EDRF and nitric oxide) on the relaxations to bradykinin and nitric oxide were examined in the normal arteries to characterize the mechanism of relaxations. The effects of a single concentration of M&B22,948 (3×10–5 M) was studied on rings left unstimulated at the optimal length (myogenic tone) and after contraction to UTP. In rings in which the time course of relaxation to M&B22,948 was studied, the time required to reach 75% of the amplitude of the plateau relaxation (T0.75) was measured.

Measurement of cGMP

A radioimmunoassay technique was used to determine the tissue content of cGMP. Rings (with or without endothelium) were initially incubated in control solution in organ chambers (5 ml) bubbled with 95% O2–5% CO2 gas mixture and kept at 37°C. After 30 minutes, the media was replaced by control solution containing a nonspecific phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX; 10–4 M), to prevent the hydrolysis of the nucleotide. After another 30-minute equilibration period, ago
nists were injected in the chambers. Preliminary experiments demonstrated that the presence of UTP in the control solution did not affect the production of cGMP; therefore, all studies were carried out without adding the contractile agent. Rings were removed from the incubation solution and immedi
ately immersed in liquid nitrogen and kept for 30 seconds. The rings were then moved into ice-cold 1N perchloric acid, homogenized with a tissue grinder, and sonicated for 5 seconds. The suspension was centrifuged (2,000g for 10 minutes), and the supernatant and the precipitate were stored frozen for
later assay of cGMP and protein, respectively. After the addition of 9 M KOH (30 µl), the thawed supernatant (150 µl) was centrifuged for 5 minutes; it then was placed on succinic anhydride (6.15-mg aliquot, lyophilized from dioxane solution), vortexed rapidly, and stored on ice. The succininated samples were checked for pH and adjusted to the range of 5.0–6.5 with KOH (9 M). The processed samples were diluted threefold with a citrate buffer (pH 6.2).

After the diluted samples were moved to assay tubes, labeled cGMP (Biomedical Technologies, Inc., Stroughton, Mass.) and anti-cGMP antibody (AMAC, Inc., Westbrook, Maine) were added and incubated for 16 hours at 4°C. To separate the bound and free fractions of labeled cGMP, a charcoal suspension was added and centrifuged (2,000g for 20 minutes at 4°C). After the supernatant was discarded, the free fraction of labeled cGMP absorbed to the charcoal was counted.

Protein was assayed with a spectrophotometric method by using a bicinchoninic acid protein assay reagent kit (Pierce, Rockford, Ill.).

**Drugs**

The following drugs were used: bradykinin (Sigma Chemical Co., St. Louis, Mo.), 8-bromo-cGMP (Sigma), diltiazem (Marion Laboratories, Inc., Kansas City, Mo.), IBMX (Sigma), methylene blue (Sigma), 2-o-proproxyphenyl-8-azapurin-6-one (M&B22,948, May and Baker, Dagenham, England), papaverine hydrochloride (Merck), and UTP (Sigma). A stock solution of IBMX (10⁻¹ M) was made in dimethyl sulfoxide (Sigma) and diluted with control solution to obtain the concentration of 10⁻⁴ M. The stock solution of M&B22,948 (7.5×10⁻³ M) was prepared in dimethyl sulfoxide; preliminary experiments showed that the effect of the solvent alone was negligible.

**Preparation of Nitric Oxide**

A glass bulb sealed with a silicon rubber injection septum was filled with nitric oxide gas from a tank (Union Carbide, Chicago, Ill.). An appropriate volume (10–1,000 µl) was aspirated with a syringe and injected into another glass gas bulb filled with 100 ml distilled water, which had been bubbled with helium for 3 hours. The dilution was adjusted to yield stock solutions of 4×10⁻⁴ M and 4×10⁻⁵ M.

**Preparation of Oxyhemoglobin**

Commercially available bovine hemoglobin (type 1, Sigma) contains a mixture of oxyhemoglobin and methemoglobin. Sodium dithionate (Na₂S₂O₄) was added to a 1 mM solution of hemoglobin in excess of 10-fold molar amount. The reducing agent was removed by dialysis in 15 l distilled water containing 0.001% EDTA for 2 hours at room temperature. The oxyhemoglobin content was determined by spectrophotometry.

**Data Analysis**

The data are expressed as mean±SEM. The amplitude of contraction to UTP and the tension at the plateau were smaller in rings without endothelium from the subarachnoid hemorrhage group (Table 1), which confirmed previous observations.¹¹,¹⁶ The maximal relaxations induced by papaverine plus diltiazem, expressed as ratio to the tension at the plateau of contraction, were comparable between the control and the subarachnoid hemorrhage groups (Table 1). Therefore, relaxations in arterial rings contracted with UTP are expressed as percent changes of the maximal relaxation in response to papaverine (3×10⁻⁴ M) plus diltiazem (10⁻⁴ M).
levels of cGMP are expressed as picomoles per milligram protein. Preliminary experiments showed no significant changes in the total amount of protein content in the basilar artery segment of comparable length after subarachnoid hemorrhage (control group, 2.10±0.29 mg/segment [n=6]; subarachnoid hemorrhage group, 1.94±0.15 mg/segment [n=6]).

For statistical comparisons of cumulative concentration–response curves, Student’s unpaired t test was used. Multiple sets of data that are mutually independent, such as kinetics of cGMP responses, were assessed using analysis of variance. Two-way factorial analysis was performed first, and when significant treatment or group effect was detected, individual sets of data were further compared using one-way analysis of variance with critical values of modified t statistics obtained by the Bonferroni method. In all cases, values of p<0.05 were considered to be statistically significant. The number of animals in each set of data is indicated by n.

Results

Mechanical Responses

Bradykinin. In the control group, bradykinin evoked concentration-dependent relaxations in rings with endothelium (Figure 1). Treatment of the rings with endothelium with oxyhemoglobin (10^{-3} M for 20 minutes) caused a contraction; the amplitude in ratio to the maximal contraction to KCl (40 mM) was 44±10% (n=6). In the presence of oxyhemoglobin, the relaxations evoked by bradykinin were abolished and converted to contractions (Figure 1, p<0.05 at 1x10^{-10} to 3x10^{-10} M, p<0.005 at 1x10^{-9} to 3x10^{-7} M). Treatment of the rings with endothelium with methylene blue (10^{-5} M for 20 minutes) caused a contraction; the amplitude in ratio to the maximal contraction to KCl (40 mM) was 40±10% (n=5). Methylene blue reduced the amplitude of relaxation significantly (Figure 1, p<0.005 at 3x10^{-9} to 3x10^{-8} M, p<0.01 at 1x10^{-7} to 3x10^{-7} M).

In the subarachnoid hemorrhage group, the relaxations to bradykinin were impaired significantly in comparison to the control group (Figure 1, p<0.05 at 3x10^{-10} to 1x10^{-9} M, p<0.005 at 3x10^{-9} to 3x10^{-7} M).

Nitric oxide. In the control group, nitric oxide caused concentration-dependent relaxations in rings without endothelium (Figure 2). Treatment of the rings without endothelium with oxyhemoglobin (10^{-5} M for 20 minutes) evoked a small contraction; the amplitude in ratio to the maximal contraction to KCl (40 mM) was 9.7±2.7% (n=6). Oxyhemoglobin abolished the relaxations caused by nitric oxide (Figure 2, p<0.005 at 3x10^{-8} to 1x10^{-7} M). Treatment of the rings without endothelium with methylene blue (10^{-5} M for 20 minutes) caused a contraction; the amplitude in ratio to the maximal contraction to KCl (40 mM) was 36±5% (n=7). Methylene blue also inhib-
During the relaxations (Figure 2, p<0.005 at 3x10^-8 to 1x10^-5 M).

In the subarachnoid hemorrhage group, the relaxations to nitric oxide were significantly smaller than those observed in the control group (Figure 2, p<0.005 at 3x10^-7 to 1x10^-5 M).

8-Bromo-cGMP. 8-Bromo-cGMP relaxed the basilar artery rings without endothelium in a concentration-dependent fashion in both groups (Figure 3). At 1x10^-5 and 3x10^-5 M, the relaxations were slightly larger (p<0.05) in the subarachnoid hemorrhage group (Figure 3).

M&B22,948. M&B22,948 (3x10^-5 M, a specific inhibitor of cGMP phosphodiesterase) caused relaxations during contractions to UTP (3x10^-6 M) or under resting conditions (myogenic tone, Figure 4). In the control group, relaxations evoked by M&B22,948 were significantly larger in rings with than in those without endothelium under either condition (Figure 4, p<0.005 during UTP contraction, p<0.005 during resting condition, analysis of variance).

In the subarachnoid hemorrhage group, the difference caused by the presence or absence of endothelium was not statistically significant. Relaxations in rings with endothelium were reduced in the subarachnoid hemorrhage group as compared with those in the control group in both conditions (Figure 4, p<0.005). During contractions to UTP, the relaxations in rings without endothelium were larger in the control group than in the subarachnoid hemorrhage group (Figure 4, p<0.01).

The time course of the relaxations induced by M&B22,948 during contractions to UTP (3x10^-6 M) was delayed in the subarachnoid hemorrhage group.

During contractions to 3x10^-6 M UTP (Figure 4), relaxations to cGMP phosphodiesterase inhibitor M&B22,948 (3x10^-5 M) in rings with endothelium (filled bars) and without endothelium (open bars) of canine basilar arteries during contraction to UTP (3x10^-6 M; left) or under basal conditions at optimal length (myogenic tone; right). Relaxations are expressed as percentage of the maximal relaxation to papaverine (3x10^-4 M) plus diltiazem (10^-4 M). Values shown are the mean±SEM (n=6). *Significant differences (p<0.05, unpaired t test) between the control and the subarachnoid hemorrhage group.

The production of cGMP

Basal levels. In the control group, the basal level of cGMP in rings with endothelium was 7.5 times higher than that in rings without endothelium (Figure 5, p<0.005, analysis of variance). The basal level of cGMP in rings with endothelium was reduced significantly in the subarachnoid hemorrhage group compared with the control group (Figure 5, p<0.005). The levels in rings without endothelium were similar in the two groups (Figure 5).

Bradykinin. In the control group, bradykinin (10^-7 M) increased the level of cGMP in rings with endothelium (p<0.05 at 30, 60, and 120 seconds compared with the basal level at 0 seconds, analysis of variance; Figure 6). The content of cGMP reached its maximum 60 seconds after stimulation and thereafter remained elevated over the next 4 minutes. At the peak, a 4.4-fold elevation from the basal level was observed (Figure 6, p<0.01). The production of cGMP was abolished by oxyhemoglobin (10^-5 M for 20 minutes, Table 2; p<0.05, analysis of variance).
FIGURE 5. Basal levels of cGMP in rings with (filled bars) and without (open bars) endothelium of canine basilar arteries. The levels are expressed as picomoles of cGMP per milligram of protein. Values are shown as the mean±SEM (n=7 unless otherwise indicated). *Significant differences between the two compared sets of data (p<0.05, two-way factorial analysis followed by Bonferroni method of one-way analysis of variance). NS, absence of statistically significant difference; SAH, subarachnoid hemorrhage group.

In the subarachnoid hemorrhage group, a similar time course of production of cGMP was observed (nonsignificant interaction term in the two-way factorial analysis of variance), but the increases in cGMP were significantly smaller than in the control group (Figure 6, p<0.05 at 30, 60, and 120 seconds, analysis of variance).

Nitric oxide. In the control group, nitric oxide (10^{-6} M) caused a marked rise in the levels of cGMP in rings without endothelium (Figure 6, p<0.05 at 20, 40, and 60 seconds compared with the basal level, analysis of variance). The maximum was reached within 40 seconds after injection of the radical, and the increase averaged 62-fold from the basal level (p<0.01). The time course of the response was more transient than that to bradykinin. Treatment of the rings with oxyhemoglobin abolished the production of cGMP (Table 2, p<0.05, analysis of variance).

In the subarachnoid hemorrhage group, the production of cGMP evoked by nitric oxide was impaired (p<0.01) in comparison to the control group, and the duration of the response was significantly shorter (p<0.001, positive interaction term in the two-way factorial analysis of variance; Figure 6).

Discussion

The findings of the present study demonstrate that 1) relaxations evoked by bradykinin in rings with endothelium and by nitric oxide in rings without endothelium are impaired after subarachnoid hemorrhage; 2) relaxations to a cGMP phosphodiesterase inhibitor, M&B22,948, in rings with or without endothelium were inhibited while those evoked by a lipophilic cGMP analogue, 8-bromo-cGMP, are maintained or even augmented after subarachnoid hemorrhage; 3) the basal production of cGMP in rings with endothelium is impaired after subarachnoid hemorrhage; and 4) increases in the level of cGMP, evoked by bradykinin in rings with endothelium or by nitric oxide in rings without endothelium, are reduced after subarachnoid hemorrhage. These observations suggest that the loss of vascular reactivity in canine basilar artery after subarachnoid hemorrhage is due, at least in part, to an impaired production of cGMP in the smooth muscle.

EDRF activates soluble guanylate cyclase in vascular smooth muscle and increases the conversion of GTP to cGMP.3–5 cGMP reacts with cGMP-dependent protein kinase(s) and causes a cascade of changes in protein phosphorylation, resulting in dephosphorylation of myosin light chain and thus leading to relaxation.21 cGMP also moderates agonist-stimulated increases in intracellular free Ca^{2+} by stimulating its extrusion via activation of Ca^{2+}-ATPase(s)22,23 and by decreasing the influx24 and also the mobilization of the activation ion from
intracellular stores. As a rule, nitrovasodilators share with EDRF the same mechanism of relaxation; they activate soluble guanylate cyclase and increase the tissue content of cGMP, thus causing endothelium-independent relaxations.

A major finding in the present study was the demonstration that the impaired endothelium-dependent (bradykinin) as well as endothelium-independent (nitric oxide) relaxations of the smooth muscle after subarachnoid hemorrhage are associated with a reduced production of cGMP. Because all measurements of cGMP were performed in the presence of an inhibitor of phosphodiesterase(s), IBMX, the changes in the levels of cGMP are likely to reflect changes in the production rather than the hydrolysis of the nucleotide.

The resting levels of cGMP in the control group were greater in rings with than in those without endothelium. This enhanced production of cGMP was inhibited by oxyhemoglobin and presumably is due to the activation of soluble guanylate cyclase in the smooth muscle by the tonic release of EDRF by endothelial cells. The presence of a basal release of EDRF by endothelial cells is supported further by the fact that oxyhemoglobin evoked an endothelium-dependent contraction in unstimulated rings. In the subarachnoid hemorrhage group, the resting levels of cGMP in rings with endothelium were reduced when compared with those in the control group, and they were similar to those in rings without endothelium. These observations suggest that after subarachnoid hemorrhage, the production of EDRF or the activation of soluble guanylate cyclase in the smooth muscle is impaired under basal conditions. This view is supported also by the fact that M&B 22,948, which prevents the hydrolysis of cGMP by phosphodiesterases, evoked relaxations in rings with endothelium both under basal conditions (myogenic tone) and after contraction by UTP; these relaxations were reduced in the subarachnoid hemorrhage group. Furthermore, the relaxations evoked by M&B 22,948 in rings without endothelium contracted by UTP were also inhibited in the subarachnoid hemorrhage group, suggesting that an altered cGMP pathway is also present at the smooth muscle level.

The endothelium-dependent relaxations evoked by bradykinin in the canine basilar artery were associated with a time-dependent production of cGMP. The relaxations were inhibited by methylene blue and were abolished by oxyhemoglobin. These observations indicate that in the canine basilar artery, as in other vascular preparations, bradykinin enhances the production of EDRF, which diffuses to the underlying smooth muscle. There, it activates soluble guanylate cyclase, leading to an enhanced production of cGMP, which then evokes relaxation. After subarachnoid hemorrhage, both the endothelium-dependent relaxation and the production of cGMP were impaired, suggesting that in the pathological model the production of EDRF or activation of soluble guanylate cyclase from the smooth muscle is altered. Bioassay experiments of canine basilar arteries with endothelium have demonstrated that the luminal release of EDRF stimulated by bradykinin and estimated indirectly by the relaxation it evoked on a coronary artery without endothelium were not altered after subarachnoid hemorrhage. However, these observations do not rule out the possibility that the abluminal release of EDRF may be reduced in the subarachnoid hemorrhage model.

In the canine basilar artery, nitric oxide evoked endothelium-independent relaxations that were associated with a time-dependent accumulation of cGMP. The relaxations were impaired markedly by methylene blue and oxyhemoglobin, indicating that they are mediated by the activation of soluble guanylate cyclase. After subarachnoid hemorrhage, both the relaxation and the production of cGMP were deficient.

High concentrations of 8-bromo-cGMP, an analogue of cGMP that permeates the cell membrane and that does not undergo hydrolysis by phosphodiesterases, evoked similar or even augmented relaxations in rings without endothelium from the subarachnoid hemorrhage group, indicating that the cGMP-dependent relaxing pathway in the smooth muscle remains functional after subarachnoid hemorrhage.

In a primate model of chronic cerebral vasospasm, the endothelium-dependent relaxations caused by histamine and the calcium ionophore A23187 were
impaired in the spastic middle cerebral arteries when compared with control arteries while the nitrovasodilator glyceryl trinitrate evoked endothelium-indepedent relaxations that were similar in both types of vessels. The fact that the relaxations evoked by nitrovasodilators are unaffected in vasospastic cerebral arteries from primates but not from canines may be due to the differences in the experimental conditions (the control and spastic arteries were taken from the same primate, whereas in the present study, they were taken from two different animals), animal species, arteries tested, and contribution of the cGMP relaxing pathway in the relaxations evoked by glyceryl trinitrate and authentic nitric oxide in primate and canine cerebral arteries, respectively.

Taken in conjunction, the present observations suggest that an altered activation of the soluble guanylate cyclase from the smooth muscle underlies the reduced endothelium-dependent and -independent relaxations mediated by nitric oxide in the canine basilar artery after subarachnoid hemorrhage. The impaired activation of the enzyme may be due to a decreased supply of the substrate (GTP) or to a decreased activity of soluble guanylate cyclase. As a factor that may affect the latter, biochemical studies demonstrate that changes in the calcium concentration affect the activity of the enzyme. Alternatively, a reduced activation of the enzyme also may be a consequence of the presence of hemoglobin in the arterial wall. In fact, oxyhemoglobin, but not methemoglobin, is able to capture nitric oxide and to compete with the heme moiety of guanylate cyclase for binding of the radical. It is likely that nitric oxide is the EDRF responsible for the activation of guanylate cyclase during endothelium-dependent relaxations. If sufficient amounts of hemoglobin from the subarachnoid clot remain within the arterial wall despite the repeated irrigations in the organ chambers and exist in the form of oxyhemoglobin despite the 1-week interval between the cisternal injection of venous blood and the experiment, then the observed loss of cGMP responses could be due to the inactivation of EDRF or nitric oxide by hemoglobin.

Acknowledgment
The authors would like to thank Mr. Robert R. Lorenz for his assistance with the drawings.

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KEY WORDS * endothelium * nitric oxide * cGMP * basilar artery * subarachnoid hemorrhage
Reduced production of cGMP underlies the loss of endothelium-dependent relaxations in the canine basilar artery after subarachnoid hemorrhage.

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Circ Res. 1992;70:248-256
doi: 10.1161/01.RES.70.2.248

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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