Impaired cerebral vasodilator responses to NO and PDE V inhibition after subarachnoid hemorrhage

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Sobey, Christopher G., and Lilly Quan. Impaired cerebral vasodilator responses to NO and PDE V inhibition after subarachnoid hemorrhage. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1718–H1724, 1999.—Subarachnoid hemorrhage (SAH) is associated with impaired nitric oxide (NO)-mediated cerebral vasodilatation. We tested the hypothesis that SAH causes alterations in the production of, hydrolysis of, or responsiveness to cGMP in the rat basilar artery in vivo. Rats were injected with saline or autologous blood into the cisterna magna. Two days later, effects of vasoactive drugs on basilar artery diameter were examined using a cranial window preparation. Vasodilator responses to ACh, sodium nitroprusside (SNP), and low concentrations (≤10⁻⁵ M) of zaprinast, an inhibitor of phosphodiesterase V (PDE V), were impaired in SAH rats (P < 0.05). In contrast, vasodilator responses to adenosine and 8-BrcGMP were similar in control and SAH rats. Vasconstrictor responses to 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one, an inhibitor of soluble guanylate cyclase, were unaffected by SAH. In the presence of zaprinast (10⁻⁵–10⁻⁴ M), responses to ACh and SNP were equivalent in control and SAH rats. Thus an increased rate of cGMP hydrolysis by PDE V may be a major factor contributing to the impairment of NO-mediated cerebral vasodilatation after SAH.

basilar artery; 1H-[1,2,4]oxadiazolo[4,3-a]quinazolin-1-one; guanosine 3',5'-cyclic monophosphate; soluble guanylate cyclase; adenosine; zaprinast; nitric oxide

AFTER ANEURYSMAL RUPTURE, exposure of cerebral arteries on the surface of the brain to the resulting blood clot causes alterations in vascular reactivity over several days. Abnormalities of cerebral artery function, such as impaired vasodilatation and increased vasoconstriction, are thought to cause major complications in subarachnoid hemorrhage (SAH) patients, including neurological deficits, stroke, and death (11, 41). There is evidence to suggest that disruption of the nitric oxide-cGMP vasodilator mechanism after SAH may contribute to many of the functional changes in these cerebral arteries, including the development of delayed vasoconstriction and impairment of vasodilator responses to endothelium-dependent agonists and nitrovasodilators (3, 38, 41).

It is well established that endothelium-dependent, nitric oxide-mediated cerebral vasodilator responses are impaired in a variety of animal models of SAH (5, 10, 14, 39) and also in patients with SAH (9, 27). This may be partly due to reduced nitric oxide release resulting from damaged endothelial cells (2, 33), but importantly, it seems that other mechanisms may also contribute. For example, numerous studies have reported that cerebral vasodilator responses to nitric oxide donor drugs are also impaired after SAH (14–16, 27, 44, 45), suggesting that responsiveness of cerebral vascular muscle to nitric oxide is altered.

In the present study we have used a rat model of SAH to investigate in further detail the mechanism(s) underlying impaired cerebral vasodilator responses to nitric oxide in vivo. Using a cranial window preparation, we measured vasodilator responses of the basilar artery and compared between control and experimental SAH animals. Our approach was to use relatively selective pharmacological agents to examine which aspects of the nitric oxide-cGMP vasodilator pathway may be abnormal in vivo following SAH.

METHODS

Male Sprague-Dawley rats [225–450 g, 316 ± 7 g (mean ± SE), n = 64] were studied. The rats were housed with a 12:12-h light/dark cycle and had access to food and water ad libitum. The study was approved by The University of Melbourne, Departments of Pharmacology and Physiology Animal Experimentation Ethics Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia.

Induction of SAH. Rats (n = 27) were anesthetized with pentobarbital sodium (50 mg/kg ip) and treated with atropine (0.5 mg/kg ip; to inhibit respiratory secretions). The rats were then intubated (tracheotomy) and mechanically ventilated with room air and placed in a supine position on a heating pad. Using aseptic technique, we cannulated the left femoral artery for the removal of blood, placed the animal in a stereotaxic device in a slight nose-down position (10°), and exposed the atlantooccipital membrane. A 27-gauge hypodermic needle was inserted 1.5 mm into the cisterna magna, and ~0.1 ml of cerebrospinal fluid (CSF) was gently aspirated. Freshly drawn autologous nonheparinized arterial blood (0.3 ml) was then injected into the CSF via the cisterna magna over ~1 min. After the injection, the animal remained in the nose-down position for 10 min with the needle still positioned in the CSF. The needle was then removed and the head incision was closed using 5–0 silk sutures. After ligation of the femoral artery, the catheter was removed and the leg incision closed. The entire procedure was completed in ~1.5 h and, when necessary, anesthesia was supplemented with pentobarbitone (10–20 mg/kg ip). Animals were fully awake 2–3 h after the surgery and were studied 2 days later.

For comparison, control animals were either similarly injected with 0.3 ml of saline (n = 9) or naive control rats (n = 28) were used. Because similar results were observed in saline-injected and naive control groups, data from the two control groups were combined (n = 37).

Cranial window preparation. Two days after the induction of experimental SAH or injection of saline, rats were again anesthetized with pentobarbitone sodium (50 mg/kg ip) for study of basilar artery reactivity. Anesthesia was supple-
ment throughout the experiment at 10–20 mg·kg⁻¹·h⁻¹ iv. A midline incision was made in the neck, a tracheostomy was performed, and the animals were mechanically ventilated with room air and supplemental O₂. Arterial blood gases were monitored and maintained within normal levels throughout the experiment (pH = 7.39 ± 0.01; PO₂ = 153 ± 5 mmHg; PCO₂ = 37 ± 1 mmHg; n = 64). A catheter was placed in the right femoral artery to measure systemic arterial blood pressure and to obtain arterial blood. The right femoral vein was cannulated for injection of supplemental anesthetic. Rectal temperature was monitored and maintained at 37–38°C with a heating pad. On occasions, gallamine triethiodide (60 mg iv) was administered to induce skeletal muscle paralysis and thus eliminate spontaneous respiratory movements. Depth of anesthesia was evaluated at least every 30 min by applying pressure to a paw and observing the effects on heart rate or blood pressure. If any changes occurred, additional anesthetic was administered.

A craniotomy was performed over the ventral brain stem, as described in detail previously (7). The cranial window was continuously suffused at 3 ml/min with artificial CSF [37–38°C; ionic composition (in mmol/l): 132 NaCl, 2.97 KCl, 3.69 d-glucose, 1.71 CaCl₂, 0.64 MgCl₂, and 22.6 NaHCO₃], which was bubbled with 95% N₂-5% CO₂ (CSF sampled from the cranial window was pH = 7.34 ± 0.01; PO₂ = 117 ± 3 mmHg; PCO₂ = 36 ± 1 mmHg; n = 64). Diameter of the basilar artery was monitored using a microscope equipped with a TV camera coupled to a video monitor and was continuously measured using a computer-based tracking program (Di- amtrak; Montech, Australia).

Experimental protocol. After an equilibration period of at least 30 min to allow for stabilization of vessel diameter and blood pressure, ACh (10⁻⁶ M) was initially applied to the cranial window to evaluate endothelial function of each preparation. Experimental vasoactive drugs were then applied topically to the basilar artery within the CSF in a cumulative manner (2–4 concentrations per drug). Drugs studied were the following: ACh, an endothelium-dependent vasodilator that stimulates production of nitric oxide; SNP, a nitric oxide donor drug; adenosine, a vasodilator that stimulates production of cGMP; zaprinast, a selective inhibitor of phosphodiesterase type V (PDE V); and 3-isobutyl-1-methylxanthine (IBMX), a nonspecific PDE inhibitor. In some experiments, we assessed vascular responses to a mixture of zaprinast plus SNP or ACh. A final concentration of 10⁻⁴ M zaprinast was the highest possible due to solubility limitations. Diameter of the basilar artery was recorded under basal conditions and during application of each concentration of drug, and the steady-state change in diameter, which was usually achieved within 3–5 min, was recorded. A recovery period of at least 15 min was allowed between applications of each drug. There was no observed effect on arterial pressure of any drug applied to the cranial window. Five to seven vasoactive drugs were studied in each rat.

Drugs. All drugs were obtained from Sigma Chemical (St. Louis, MO), except ODQ (from Sapphire, Australia). All drugs were prepared in 0.9% saline, except zaprinast, which was prepared as a 10⁻³ M stock solution in 5% DMSO-95% saline, and ODQ, which was prepared as a 3 × 10⁻² M stock solution in 100% DMSO. The vehicle for zaprinast and ODQ had no effect on basilar artery diameter when applied alone in equivalent concentrations (i.e., <0.5% DMSO).

Data analysis. Vascular responses are presented as percentage change in basilar artery diameter over baseline diameter and are expressed as means ± SE. Student’s unpaired or paired t-tests were used, as appropriate, to compare data. A P value <0.05 was considered significant.

RESULTS

Mean arterial pressure was similar in control (93 ± 2 mmHg) and SAH rats (92 ± 2 mmHg). Baseline diameter of the basilar artery in control rats was 233 ± 4 µm (range = 174–284 µm, median = 234 µm), which was slightly but significantly larger than the artery diameter in SAH rats (219 ± 5 µm; range = 162–262 µm, median = 229 µm, P < 0.05 vs. control).

Basilar artery responses to ACh, SNP, and adenosine. ACh diluted the basilar artery in a concentration-dependent manner (Fig. 1). Vasodilator responses to ACh were significantly smaller in SAH rats (Fig. 1). SNP produced concentration-dependent dilatation of the basilar artery under control conditions (Fig. 2) that was also smaller in SAH rats (Fig. 2). Dilator responses of the basilar artery to adenosine, an activator of the adenate cyclase-cAMP pathway, were concentration dependent and similar in control and SAH rats (Fig. 3).

Vasodilator responses to 8-BrcGMP, ODQ, zaprinast, and IBMX. 8-BrcGMP, a stable analog of cGMP that is resistant to hydrolysis by PDEs, produced concentration-dependent vasodilator responses that were similar in control and SAH rats (Fig. 4).

ODQ produced concentration-dependent constriction of the basilar artery, consistent with inhibition of activity of sGC and decreased basal effects of cGMP. The response to ODQ was not altered after SAH (Fig. 5).

Zaprinast, an inhibitor of PDE V, which is a major enzyme responsible for the hydrolysis of cGMP (1, 23), produced concentration-dependent dilatation of the basilar artery in control rats (Figs. 6A and 7). ODQ (10⁻⁵ M) significantly inhibited vasodilator responses to zaprinast (Fig. 6A), indicating that dilatation of the basilar artery by zaprinast is dependent on basal activity of sGC. ODQ had no effect on vasodilator responses to 8-BrcGMP, confirming that this response is independent of sGC activation (Fig. 6B).

In SAH rats, vasodilator responses to low concentrations (3 × 10⁻⁶–3 × 10⁻⁴ M) of zaprinast were significantly impaired, but responses to higher concentrations (3 × 10⁻³
10^{-5}–10^{-4} M) were not different between control and SAH groups (Fig. 7). This represents a rightward shift in the zaprinast concentration-response curve, with no apparent change to the response to the highest concentration (Fig. 7).

Dilator responses of the basilar artery to IBMX, a nonselective inhibitor of PDEs, were concentration dependent and similar in control and SAH rats (Fig. 8).

Effect of zaprinast on cerebral vasodilator responses to SNP and ACh. When ACh or SNP was applied in the presence of zaprinast (10^{-2} M or 10^{-4} M), dilator responses in SAH rats were restored to levels equivalent to responses in control rats (Fig. 7). Reponses to ACh and SNP (especially lower concentrations) were augmented in the presence of zaprinast, suggesting that PDE V activity normally modulates this vasodilator response.

DISCUSSION

There are several major findings of this study. First, vasodilator responses of the basilar artery to SNP, ACh, and zaprinast (an inhibitor of PDE V), agents that elicit production and/or accumulation of cGMP in vascular muscle, were selectively impaired after SAH. Second, vasodilator responses to 8-BrcGMP, a PDE-resistant analog of cGMP, were similar in control and SAH rats. Third, application of high concentrations of zaprinast, to substantially if not completely inhibit PDE V activity, restored to control levels the vasodilator responses to SNP and ACh after SAH. Fourth, vasoconstrictor responses to ODQ, a selective inhibitor of sGC, were normal after SAH. Together, these data are consistent with an increased rate of hydrolysis of cGMP by PDE V in the basilar artery contributing to the impairment of nitric oxide-mediated cerebral vasodilation after SAH.

Effects of SAH on cerebral vasodilator responses to nitric oxide and cGMP. We found that vasodilation in response to both ACh, an endothelium-dependent vasodilator, and SNP, a nitric oxide donor, was reduced after SAH, confirming previous findings (5, 10, 14, 27, 39). In the rat basilar artery, vasodilator responses to ACh appear to be exclusively mediated by the release of endothelium-derived nitric oxide (6, 17, 19, 24, 35). Thus SAH impairs vasodilator responses of the basilar artery to both endogenous (ACh) and exogenous (SNP) nitric oxide. Although this is not a universal finding (10, 21, 31, 43), several others (14–16, 27, 44, 45) have also reported that cerebral vasodilator responses to nitric oxide are impaired after SAH. This phenomenon is significant because it indicates that the impairment of endothelium-dependent cerebral vasodilation observed in this and some other models of SAH (14–16, 27, 44, 45) and in patients with SAH (27) is not simply...
due to endothelial cell dysfunction but may at least partly be due to decreased responsiveness of vascular muscle to nitric oxide. Furthermore, we found that dilator responses of the basilar artery to adenosine, which stimulates production of cAMP by adenylate cyclase, were preserved after SAH, consistent with previous reports using agents that elevate cAMP (29, 40). This finding also confirms that in the rat model of SAH, impairment of cerebral vasodilatation mediated by the nitric oxide-cGMP pathway is relatively selective.

Role of PDE V in cerebral artery responses to nitric oxide and cGMP after SAH. PDEs hydrolyze cyclic nucleotides (i.e., cGMP and cAMP) and thus modulate their vasorelaxant effects in vascular muscle, and PDE V is an important enzyme for the hydrolysis of cGMP (1, 23). Zaprinast, which is thought to be a relatively selective inhibitor of PDE V (1, 23), causes intracellular accumulation of cGMP and has been reported to elicit dilatation of dog cerebral arteries in vitro (13, 15, 30) and of cerebral arterioles of piglets in vivo (32). Because topical application of very high concentrations (≥10^{-4} M) of zaprinast was reported to increase levels of both cGMP and cAMP in the CSF of piglets (32), caution should be used in interpreting in vivo cerebral vasodilator responses to this agent. However, in the present study we found that dilatation of the rat basilar artery in vivo in response to zaprinast is inhibited by ODQ, suggesting that vasodilatation by zaprinast was mainly dependent on the generation (and accumulation) of cGMP by sGC.

A new finding of the present study is that after SAH cerebral vasodilator responses to zaprinast are impaired in vivo. Compared with responses in control rats, the concentration-response curve to zaprinast (3×10^{-6}–10^{-4} M) was shifted to the right after SAH. Thus at low concentrations (≤10^{-5} M), responses to zaprinast were reduced in SAH rats, whereas at higher concentrations responses were similar in control and SAH groups. Although we could not test responses to even higher concentrations (>10^{-4} M) of zaprinast because of solubility limitations, the data suggest that the maximum response to the PDE V inhibitor is probably not different between control and SAH groups. By contrast, vasodilator responses to 8-BrcGMP, a PDE-resistant analog of cGMP (20), were equivalent in control and SAH rats. Thus in these experiments SAH appears to selectively impair vasodilatation mediated by endogenous, PDE-susceptible cGMP. The finding that cerebral vasodilator responses to 8-BrcGMP are preserved after SAH is consistent with previous observations in dogs (15) and rats (39, 44) and suggests that the abnormality in responsiveness to nitric oxide is unlikely to be due to alterations in function of intracellular targets of cGMP. This latter finding has been further interpreted as reflecting impaired activity of sGC in cerebral vascular muscle after SAH (15, 39, 44).
Although the present evidence is indirect, a novel interpretation of our findings is that there may be increased activity of PDE V, rather than (or in addition to) decreased activity of sGC, in the basilar artery after SAH. If this were the case, higher concentrations of zaprinast would be required in SAH than control rats to effectively inhibit enzyme activity and allow similar levels of cGMP accumulation and vascular relaxation, as was observed. Kim et al. (15) found that the vasorelaxant response of dog basilar artery rings to a single concentration (3 \times 10^{-5} M) of zaprinast (M & B22,948) was impaired after SAH and concluded that cGMP production (and hence accumulation) was reduced. However, our data indicate that greater (and perhaps near maximal) inhibition of PDE V using a higher concentration (10^{-4} M) of zaprinast achieves a larger vasodilator response that is equivalent in control and SAH rats, as might be expected if the reduced responsiveness to nitric oxide after SAH is in fact due to increased activity of PDE V.

To test for further evidence that vasodilator responses to nitric oxide are impaired after SAH because of increased hydrolysis of cGMP by PDE V, we performed additional experiments in which ACh and SNP were applied to the cranial window in the presence of relatively high concentrations (10^{-5} and 10^{-4} M) of zaprinast. Using this approach, we sought to examine responses to endogenous cGMP in the absence of (or during very low rates of) hydrolysis by PDE V. We found that responses to both ACh and SNP were augmented by zaprinast, suggesting that PDE V activity normally modulates cerebral vasodilator responses to nitric oxide. Moreover, responses to ACh and SNP were equivalent in control and SAH rats when applied to the basilar artery in the presence of zaprinast. Thus the data support the conclusion that in this model of SAH decreased stability of endogenous cGMP due to increased PDE V activity may largely account for why the vasodilator responses to SNP, ACh, and low concentrations of zaprinast are impaired, whereas the response to a PDE-resistant analog of cGMP (8-BrcGMP) is preserved. Hence, although morphological damage to endothelial cells may occur after SAH (2, 33), our data can be explained even if endothelial dysfunction did not occur and are thus compatible with the findings that endothelial nitric oxide synthase expression is unchanged (12) and endothelial release of nitric oxide is normal (14) in the basilar artery after SAH.

Vasodilator responses to the nonselective PDE inhibitor IBMX were similar in control and SAH animals. This probably indicates that PDE-mediated hydrolysis of cAMP predominates over that of cGMP in the basilar artery (42), such that total basal PDE activity may remain similar in control and SAH animals. Hence, our finding that the vasodilator effect of adenosine was unaltered after SAH, suggesting that PDE-mediated mechanisms were preserved, would be consistent with the effect of IBMX being due predominantly to the hydrolysis of cAMP in the basilar artery. Moreover, the impairment of vasodilator responses to zaprinast suggests that SAH may selectively interfere with PDE V activity and that this may have significant consequences for regulation of vascular tone.

Role of sGC activity after SAH. Another possible explanation for impaired cGMP-dependent responses is that activity of sGC is reduced in the basilar artery after SAH, as has been suggested previously (5, 12, 15, 22, 25, 26, 40). ODQ is a selective inhibitor of sGC (8),
that almost completely abolishes the responses of cerebral vessels to ACh and SNP (28, 34, 36, 37). We reasoned that if basal production of cGMP by sGC was reduced after SAH in the present experiments, vasoconstrictor responses to ODQ (due to removal of vasodilator effects of cGMP produced under basal conditions) would be impaired. However, we found no difference in responses to ODQ between control and SAH rats, suggesting that modulation of arterial tone by basal cGMP generation was similar in the two groups. Although it remains possible that significant impairment of basal sGC activity does occur in experimental models of SAH in which marked vasospasm develops (12, 15, 22, 25, 26), the present findings with ODQ suggest that no change to basal sGC activity is apparent in this model (4, 39). Importantly, the data provide functional evidence that after SAH vasodilator responses to nitric oxide in vivo may also be reduced due to factors downstream of cGMP production in cerebral vascular muscle. We also cannot exclude the possibility that there is a reduced capacity for cGMP generation above basal levels by sGC when stimulated by nitric oxide after SAH. If this were the case, however, we would not expect coapplication of zaprinast to equalize responses to ACh and SNP in control and SAH rats, as was observed here. Similarly, if the impaired vasodilator responses in this study were due to inactivation of nitric oxide by oxyhemoglobin in red blood cells trapped in the subarachnoid space (5, 18), it is unlikely that responses would be normalized using a PDE V inhibitor.

Peak delayed constriction of the basilar artery occurs 2 days after SAH in the rat model used here (4) and, as our present data confirm, when studied using the cranial window method baseline artery diameter is typically ~5–10% smaller in SAH than in control rats (39). However, despite the lack of development of profound vasospasm, the present data also confirm that marked functional changes are present in the basilar artery and suggest that the model is a valid and useful approach to investigate in vivo altered mechanisms of cerebral vascular reactivity after SAH. Information regarding the actual mediator(s) involved remains controversial, but there is evidence that hemoglobin and also reactive oxygen species released from the aging clot might be key factors leading to delayed cerebrovascular dysfunction after SAH (3, 38, 41).

In summary, the results of this study demonstrate that SAH produces selective impairment of nitric oxide-mediated dilatation of the rat basilar artery in vivo. This impairment does not appear to be due to decreased vascular production of or responsiveness to cGMP. Rather, the data are consistent with an increased rate of hydrolysis of cGMP by PDE V in the basilar artery after SAH, and it seems possible that this abnormality represents the major cause of the impaired nitric oxide/cGMP-mediated vasodilator responses in this model. The finding that impaired ACh- and SNP-induced cerebral vasodilator responses can be fully restored after SAH by using a PDE V inhibitor is novel and indicates that inhibition of PDE activity may be a useful approach in SAH therapy.

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