Isoflurane does not further impair microvascular vasomotion in a rat model of subarachnoid hemorrhage

[L'isoflurane ne produit pas d'altération subséquente sur la vasomotricité microvasculaire d'un modèle d'hémorragie sous-arachnoïdienne chez le rat]

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Purpose: Since isoflurane is known to attenuate endothelium-dependent dilation (EDD) in normal cerebral arterioles, we examined whether the anesthetic has a similar effect and further impairs EDD in vessels exposed to SAH.

Methods: Autologous blood was introduced in the subarachnoid space and the parietal lobe harvested. Control animals were sacrificed without introduction of blood. The response of microvessels to the endothelium-dependent dilator adenosine diphosphate (ADP) 10^{-9} – 10^{-4} M, the endothelium-independent dilator nitroprusside 10^{-9} – 10^{-4} M, and ET-1 10^{-13} – 10^{-8} M was measured by videomicroscopy in the presence of 0–2 minimum alveolar concentration (MAC) of isoflurane.

Results: Isoflurane attenuated EDD to ADP in control vessels [$66 \pm 5\%$ (control) vs $27 \pm 11\%$ (2 MAC) dilation to ADP 10^{-4} M, $P < 0.05$]. Although SAH was associated with reduced dilation to ADP, exposure to isoflurane did not further impair dilation to ADP after SAH [$26 \pm 3\%$ (SAH) vs $21 \pm 5\%$ (SAH/2 MAC) dilation to ADP 10^{-4} M, $P = \text{NS}$]. Dilation to nitroprusside was not affected by isoflurane or SAH. Constriction to ET-1 was reduced by 2 MAC of isoflurane [$21 \pm 1\%$ (control) vs $13 \pm 5\%$ (2 MAC) constriction to ET-1 10^{-8} M, $P < 0.05$], but not by 1 MAC of isoflurane in control vessels. Constriction to ET-1 was greatly attenuated by 1 or 2 MAC of isoflurane after SAH [$32 \pm 5\%$ (SAH) vs $18 \pm 4\%$ (SAH/2 MAC) constriction to ET-1 10^{-8} M, $P < 0.05$].

Conclusion: In rats, isoflurane does not further impair EDD after SAH and modulates the constrictive response to ET-1. Such an effect of isoflurane would not predispose the SAH-exposed vessels to vasospasm.

Objectif : L'isoflurane est connu pour atténuer la dilatation dépen-

dante de l'endothélium (DDE) dans les artérioles cérébrales normales. Nous avons vérifié si l'anesthésique a un effet similaire, et s'il affecte davantage la DDE, dans les vaisseaux sanguins exposés à l'hémorragie sous-arachnoïdienne (HSA).

Méthode : Du sang autologue a été introduit dans l'espace sous-arachnoïdien et le lobe pariétal a été prélevé. Les animaux témoins ont été sacrifiés sans introduction de sang. La réaction des microvaisseaux à 10^{-9} – 10^{-4} M d'adénosine diphosphate (ADP), à 10^{-9} – 10^{-4} M de nitroprussiate (deux dilateurs dépendants de l'endothélium) et à 10^{-13} – 10^{-8} M de ET-1 a été mesurée par vidéomicroscopie en présence d'une concentration alvéolaire minimale (CAM) de 0–2 d'isoflurane.

Résultats : L'isoflurane a diminué la DDE liée à l'ADP dans les vaisseaux témoins [$66 \pm 5\%$ (témoin) vs $27 \pm 11\%$ (2 CAM) de dilatation à 10^{-4} M d'ADP, $P < 0,05$]. Bien que l'HSA soit associée à une dilatation réduite liée à l'ADP, l'exposition à l'isoflurane n'a pas accentué la dilatation liée à l'ADP à la suite d'une HSA [$26 \pm 3\%$ (HSA) vs $21 \pm 5\%$ (HSA/2 CAM) de dilatation à 10^{-4} M d'ADP, $P = \text{NS}$]. La dilatation liée au nitroprussiate n'a pas été modifiée par l'isoflurane ou l'HSA. La constriction liée à l'ET-1 a été réduite par 2 CAM d'isoflurane [$21 \pm 1\%$ (témoin) vs $13 \pm 5\%$ (2 CAM) de constriction à 10^{-8} M d'ET-1, $P < 0,05$], mais non par 1 CAM d'isoflurane dans les vaisseaux témoins. La constriction liée à l'ET-1 a été grandement atténuée par 1 ou 2 CAM d'isoflurane après une HSA [$32 \pm 5\%$ (HSA) vs $18 \pm 4\%$ (HSA/2 CAM) de constriction à 10^{-8} M d'ET-1, $P < 0,05$].

Conclusion : Chez les rats, l'isoflurane ne produit pas d'altération subséquente de la DDE à la suite d'une HSA et il module la réaction constrictive liée à l'ET-1. Cet effet de l'isoflurane ne prédisposerait pas au vasospasme les vaisseaux exposés à l'HSA.

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THE leading causes of mortality and morbidity after subarachnoid hemorrhage (SAH) are the immediate effects of SAH itself, rebleeding, and cerebral ischemia and infarction from cerebral vasospasm; and the one-month mortality rate from SAH approaches 50% in the U.S.¹ In humans, cerebral vasospasm is believed to be biphasic, with both an acute phase occurring within hours and a delayed phase usually beginning two to four days after SAH and peaking on about day seven.^{2,3} SAH-associated vasospasm occurs not only in the large cerebral conduit arteries, but also in the cerebral microvessels. Cerebral microvascular vasospasm may actually be more important in producing cerebral ischemia than conduit vessel vasospasm.⁴ In our previous study using a rat model of SAH,⁵ we demonstrated that cerebral arterioles after SAH have endothelial dysfunction that could contribute to vasospasm. As a result, cerebral arterioles after SAH demonstrated attenuated response to the endothelium-dependent dilator adenosine diphosphate (ADP) and accentuated constriction to endothelin-1 (ET-1). We showed further that such a dysfunction might be based on reduced protein expression of endothelial nitric oxide synthase (eNOS).

Studies from multiple laboratories have shown previously that inhalational anesthetics cause attenuation of endothelium-dependent vasodilation in various non-diseased vascular beds including the cerebral microcirculation.⁶⁻⁹ In this study, therefore, we examined whether the inhalational anesthetic isoflurane may have an additive effect on endothelial dysfunction of cerebral microvessels after SAH.

Methods

With Institutional Animal Care and Use Committee approval, Wistar rats of either sex, weighing 250–300 g, were anesthetized by injecting 40 mg·kg⁻¹ ketamine and 5 mg·kg⁻¹ xylazine intraperitoneally. Our rat model of SAH has been previously described.⁵ Briefly, a polyethylene catheter (PE-10) was inserted percutaneously into the cisterna magna (CM) and was secured in place. After two days of recovery, lack of gross wound infection was noted and normal neurological function was verified. The animal was then re-anesthetized with ketamine and xylazine as before. A femoral arterial catheter was placed for blood sampling. SAH was modeled by withdrawing 0.3 mL of cerebrospinal fluid from the CM and injecting 0.3 mL of fresh non-heparinized autologous blood into the CM slowly over a ten-minute period with the animal in a 20° head-down position, as described by Cole *et al.*¹⁰ The animal was allowed to breathe spontaneous-

ly and kept in the head-down position. Twenty minutes after subarachnoid injection of blood, at a time known to coincide with maximal vasospasm,¹⁰ femoral arterial blood was sampled for blood gas analysis and the animal was euthanized for harvest of the brain tissue, which was quickly placed in a cold (4°C) modified Krebs solution (120 mM NaCl, 5.9 mM KCl, 11.1 mM dextrose, 25 mM NaHCO₃, 1.2 mM NaH₂PO₄, 1.2 mM MgSO₄, and 2.5 mM CaCl₂). At necropsy, proper positioning of the polyethylene tubing in the CM and spread of the subarachnoid blood over the surface of the cerebral cortex were verified. Control animals received neither the percutaneous catheter into the CM nor subarachnoid blood injection.

Arterioles of diameters of about 100 µm were dissected from the parietal lobes of the SAH and control animals. Each vessel was placed in a vessel chamber, cannulated with dual micropipettes measuring 50–75 µm in diameter, and secured with a 10–0 suture. The vessel was continuously bathed with modified Krebs buffer, gassed with 95% O₂–5% CO₂ mixture, and maintained at 36.5–37.5°C and pH of 7.35–7.45. PO₂ in the vessel chamber exceeded 400 mmHg. Because the vessel was studied in a no-flow state, pressure in the micropipettes was maintained at 40 mmHg to provide distention. The vessel was visualized and its internal lumen diameter was measured and recorded by videomicroscopy, as previously described.¹¹ Stability of similarly prepared vessel preparations over at least 2.5 hr has been demonstrated previously.¹¹

Each vessel was equilibrated at 37°C for 30 min in the vessel chamber and the baseline internal diameter (D_{baseline}) was measured. The vessel was then precontracted with the thromboxane analogue U46619 1 µM for five minutes, since the response of the cerebral vessels to U46619 was previously demonstrated not to be influenced by SAH.⁵ The vessel was then subjected to isoflurane 0, 1, or 2 minimum alveolar concentration (MAC; 1 MAC = approximately 1.2% in rats)¹² by adding the anesthetic to the 95% O₂–5% CO₂ mixture bubbling the Krebs buffer solution, using an in-line bubble-through vaporizer. In a preliminary experiment, using gas chromatography (GC), it took less than ten minutes for isoflurane to reach a steady state concentration after it was introduced in the vessel chamber.¹¹ The anesthetic content in the gas mixture was monitored continuously using a Rascal II Gas Analyzer (Ohmeda, Salt Lake City, UT, USA.), calibrated with industrial standards. Previously we demonstrated by GC analysis that in our experimental preparation, the millimolar concentration and partial pressure of isoflurane in the vessel chamber reflected its concentration in the gas

mixture bubbled into the buffer solution.¹¹

The diameter obtained after U46619 and isoflurane (or U46619 alone in case of a vessel without isoflurane exposure) was considered as the constricted diameter (D_{const}). At least 15 min after introduction of isoflurane, the vessel was subjected to increasing concentrations of (a) the endothelium-dependent dilator ADP 10^{-9} – 10^{-4} M or (b) the endothelium-independent dilator sodium nitroprusside (SNP) 10^{-9} – 10^{-4} M. At each concentration of the dilator, the internal diameter was measured (D_{dil}) and % dilation calculated as follows:

$$\% \text{ dilation} = (D_{\text{dil}} - D_{\text{const}}) / (D_{\text{baseline}} - D_{\text{const}}) \times 100$$

At the end of each experiment, the vessel chamber was flushed with fresh Krebs buffer and the vessel re-equilibrated at 37°C. Potassium chloride (KCl) was then added to a final concentration of 100 mM, and the internal lumen diameter was measured. Functional endothelium integrity was tested by measuring the response to ADP 10^{-5} M. Only those vessels that constricted by at least 15% to KCl and dilated at least 15% to ADP at the end of each experiment were consid-

ered viable and included for data analysis.

To examine the effect of isoflurane on ET-1-mediated vasoconstriction, additional SAH and control vessels were equilibrated at 37°C. Following exposure to isoflurane 0, 1, or 2 MAC for 15 min, the vessel diameter was measured and this value was taken as the D_{baseline} . Each vessel was then subjected to ET-1 10^{-13} – 10^{-8} M. Vessel diameter was measured at each concentration of the constrictor (D_{const}) and % constriction calculated from the D_{baseline} :

$$\% \text{ constriction} = (1 - D_{\text{const}} / D_{\text{baseline}}) \times 100$$

Vessel viability was tested as above.

No animal contributed more than one vessel to any one experimental group. Therefore, *n* for each group represents the number of animals as well as the number of vessels. All data are presented as mean $\pm$ SD. Presence of a concentration-dependent vasomotion to a vasodilator or a constrictor was tested by one-way analysis of variance (ANOVA) (linear contrast). The effects of SAH or isoflurane on concentration response curves to the various vasomotor agents tested were analyzed by two-way ANOVA with a repeated measures factor, with posthoc multiple pairwise comparison (Neuman-Keuls) and stratified *z* tests to identify the concentrations where the differences in

FIGURE 1 Percent dilation of U46619-precontracted cortical microvessels *vs* logarithm of concentration of the endothelium-dependent dilator adenosine diphosphate (ADP). A) Dilation to ADP was significantly attenuated by 1 or 2 minimum alveolar concentration (MAC) of isoflurane in the control vessels. $*P < 0.05$ *vs* control. B) Dilation to ADP was significantly attenuated by exposure to subarachnoid hemorrhage (SAH), but there was no further significant attenuation by 1 or 2 MAC of isoflurane. $*P < 0.05$ *vs* SAH without isoflurane.

FIGURE 2 Percent dilation of U46619-precontracted cortical microvessels *vs* logarithm of concentration of the endothelium-independent dilator nitroprusside. Dilation to nitroprusside was not significantly affected by 1 or 2 minimum alveolar concentration (MAC) isoflurane either in the control vessels (a) or in vessels exposed to subarachnoid hemorrhage (SAH) (b).

TABLE I Percent dilation of rat cerebral microvessels to ADP

Concentration of ADP (M)	Control			After subarachnoid hemorrhage		
	Concentration of isoflurane (MAC)			Concentration of isoflurane (MAC)		
	0	1	2	0	1	2
10 ⁻⁹	5 ± 2	4 ± 2	5 ± 2	4 ± 2	4 ± 1	3 ± 2
10 ⁻⁸	12 ± 3	10 ± 3	9 ± 3	8 ± 4	12 ± 3	7 ± 4
10 ⁻⁷	26 ± 6	17 ± 5*	14 ± 3*	13 ± 5#	15 ± 3	11 ± 5
10 ⁻⁶	42 ± 5	23 ± 8*	18 ± 6*	19 ± 8#	20 ± 6	15 ± 5
10 ⁻⁵	58 ± 5	30 ± 6*	24 ± 8*	22 ± 10#	22 ± 4	15 ± 5
10 ⁻⁴	67 ± 4	29 ± 6*	27 ± 11*	25 ± 11#	26 ± 4	19 ± 7

Data are expressed as mean ± SD. ADP = adenosine diphosphate; MAC = minimum alveolar concentration. * $P < 0.05$ vs 0 MAC of isoflurane in the same experimental group. # $P < 0.05$ vs control at the same concentration of isoflurane.

TABLE II Percent dilation of rat cerebral microvessels to nitroprusside

Concentration of nitroprusside (M)	Control			After subarachnoid hemorrhage		
	Concentration of isoflurane (MAC)			Concentration of isoflurane (MAC)		
	0	1	2	0	1	2
10 ⁻⁹	6 ± 3	4 ± 1	7 ± 4	5 ± 2	4 ± 2	4 ± 3
10 ⁻⁸	15 ± 3	12 ± 2	18 ± 7	12 ± 3	10 ± 2	11 ± 3
10 ⁻⁷	29 ± 5	25 ± 4	28 ± 7	26 ± 5	19 ± 2	21 ± 3
10 ⁻⁶	48 ± 7	41 ± 5	44 ± 7	46 ± 8	34 ± 5	39 ± 4
10 ⁻⁵	68 ± 8	60 ± 8	57 ± 9	64 ± 7	57 ± 3	59 ± 3
10 ⁻⁴	82 ± 6	76 ± 6	73 ± 10	77 ± 5	76 ± 2	73 ± 6

Data are expressed as mean ± SD. MAC = minimum alveolar concentration.

TABLE III Percent constriction of rat cerebral microvessels to endothelin-1

Concentration of endothelin-1 (M)	Control			After subarachnoid hemorrhage		
	Concentration of isoflurane (MAC)			Concentration of isoflurane (MAC)		
	0	1	2	0	1	2
10 ⁻¹³	2 ± 1	4 ± 1	2 ± 2	3 ± 1	3 ± 1	2 ± 1
10 ⁻¹²	6 ± 2	6 ± 2	4 ± 3	7 ± 2	6 ± 1	4 ± 2
10 ⁻¹¹	9 ± 2	10 ± 2	7 ± 4	11 ± 2#	9 ± 2*	7 ± 2*
10 ⁻¹⁰	12 ± 3	12 ± 3	9 ± 4	18 ± 2#	11 ± 2*	9 ± 2*
10 ⁻⁹	15 ± 3	15 ± 3	10 ± 5	25 ± 2#	15 ± 3*	13 ± 3*
10 ⁻⁸	19 ± 4	18 ± 3	13 ± 5*	32 ± 5#	19 ± 3*	18 ± 4*

Data are expressed as mean ± SD. MAC = minimum alveolar concentration. * $P < 0.05$ vs 0 MAC of isoflurane in the same experimental group. # $P < 0.05$ vs control at the same concentration of isoflurane.

response were significant. Where appropriate, two-tailed Student's *t* test was used to compare the means of two groups. $P < 0.05$ was considered significant. All statistics were calculated using True Epistat software (Epistat Services, Richardson, TX, USA).

Results

Blood gases obtained just prior to harvest of the brain tissue demonstrated no significant respiratory depression, hypoxia, or acidosis.

To examine the dilatory response of the isolated cerebral microvessels, they were precontracted with U46619 1 µM and then exposed to isoflurane 0–2

MAC, prior to subjecting them to increasing concentrations of selected dilators. No significant change in internal diameter of the U46619-precontracted vessel was noted after steady state concentrations of isoflurane were obtained.

The dilatory response of the cerebral microvessels to the endothelium-dependent dilator ADP was attenuated by either 1 or 2 MAC of isoflurane (control with no isoflurane: $n = 9$, $D_{\text{baseline}} = 93 \pm 6$ µm; control with isoflurane 1 MAC: $n = 8$, $D_{\text{baseline}} = 102 \pm 8$ µm; control with isoflurane 2 MAC: $n = 9$, $D_{\text{baseline}} = 101 \pm 6$ µm; $P < 0.001$ for either 1 or 2 MAC vs 0 MAC; $P = \text{NS}$ between 1 and 2 MAC; Figure 1a; Table I). Although

FIGURE 3 Percent constriction of cortical microvessels *vs* logarithm of concentration of endothelin-1. A) Constriction to endothelin-1 was significantly attenuated by 2 minimum alveolar concentration (MAC) of isoflurane, but not by 1 MAC of isoflurane in control vessels. * $P < 0.05$ *vs* control. B) Constriction to endothelin-1 was significantly increased in subarachnoid hemorrhage (SAH)-exposed vessels compared to control, but isoflurane 1 or 2 MAC significantly modulated constriction to endothelin-1 in SAH-exposed vessels. * $P < 0.05$ *vs* control.

vessels after SAH showed attenuated response to ADP compared to control ($P < 0.001$), there was no further attenuation of SAH vessels by either 1 or 2 MAC of isoflurane ($P = \text{NS}$) (SAH with no isoflurane: $n = 7$, $D_{\text{baseline}} = 96 \pm 9 \mu\text{m}$; SAH with isoflurane 1 MAC: $n = 6$, $D_{\text{baseline}} = 100 \pm 7 \mu\text{m}$; SAH with isoflurane 2 MAC: $n = 8$, $D_{\text{baseline}} = 103 \pm 7 \mu\text{m}$; Figure 1b; Table I).

The dilatory response of the cerebral microvessels to the endothelium-independent dilator SNP was not affected by either 1 or 2 MAC of isoflurane with or without SAH (control with no isoflurane: $n = 7$, $D_{\text{baseline}} = 92 \pm 6 \mu\text{m}$; control with isoflurane 1 MAC: $n = 9$, $D_{\text{baseline}} = 96 \pm 6 \mu\text{m}$; control with isoflurane 2 MAC: $n = 8$, $D_{\text{baseline}} = 96 \pm 8 \mu\text{m}$; SAH with no isoflurane: $n = 7$, $D_{\text{baseline}} = 89 \pm 8 \mu\text{m}$; SAH with isoflurane 1 MAC: $n = 6$, $D_{\text{baseline}} = 100 \pm 7 \mu\text{m}$; SAH with isoflurane 2 MAC: $n = 8$, $D_{\text{baseline}} = 92 \pm 6 \mu\text{m}$; $P = \text{NS}$; Figures 2a and b; Table II).

The constrictive response of the cerebral microvessels to ET-1 was not affected by 1 MAC of isoflurane ($P =$

NS), but was attenuated by 2 MAC of isoflurane ($P < 0.05$; control with no isoflurane: $n = 9$, $D_{\text{baseline}} = 99 \pm 9 \mu\text{m}$; control with isoflurane 1 MAC: $n = 9$, $D_{\text{baseline}} = 90 \pm 8 \mu\text{m}$; control with isoflurane 2 MAC: $n = 8$, $D_{\text{baseline}} = 91 \pm 9 \mu\text{m}$; Figure 3a; Table III). Whereas constriction to ET-1 was increased after SAH compared to control ($P < 0.001$), constriction to ET-1 after SAH was attenuated by either 1 or 2 MAC of isoflurane ($P < 0.001$ each *vs* no isoflurane after SAH; SAH with no isoflurane: $n = 6$, $D_{\text{baseline}} = 104 \pm 11 \mu\text{m}$; SAH with isoflurane 1 MAC: $n = 6$, $D_{\text{baseline}} = 92 \pm 7 \mu\text{m}$; SAH with isoflurane 2 MAC: $n = 7$, $D_{\text{baseline}} = 91 \pm 7 \mu\text{m}$; Figure 3b; Table III).

Discussion

The main findings of the present study are that (a) although isoflurane causes attenuation of EDD in normal cerebral arterioles, it does not have a similar effect in the microvessels with endothelial dysfunction after SAH and (b) accentuation of constriction to ET-1 seen after SAH is attenuated by isoflurane. These effects of isoflurane might tend to have a protective effect against microvascular spasm following SAH.

There are both *in vitro* and *in vivo* studies that suggest that isoflurane is a cerebral vasodilator. Farber *et al.*¹³ found that, in precontracted intraparenchymal cortical and hippocampal arterioles of rats, both isoflurane and halothane dilated the vessels in a dose-dependent manner, but that the vasodilatory effect of isoflurane was significantly less in the cortex than in the hippocampus. Clinically, systemic hypotension and consequent reduction in cerebral perfusion pressure (CPP) produced by isoflurane in patients undergoing craniotomy are not associated with any reduction in cerebral blood flow (CBF).^{14,15} Presumably, vasodilation produced by isoflurane compensates for the decrease in CPP to maintain CBF. This effect is present even following SAH.¹⁵ With isoflurane-induced vasodilation, there is a shift in the relationship between cerebral metabolic rate and CBF, so that for any given level of cerebral glucose metabolism¹⁶ or oxygen consumption,¹⁷ there is an increased level of CBF in the presence of isoflurane.

The role of endothelium in the observed vasomotor effects of isoflurane is complex and controversial. On the one hand, it has been shown that NOS inhibition can reduce CBF during isoflurane anesthesia¹⁸ and can block an increase in CBF by isoflurane,¹⁹ implying a role of NO in isoflurane-mediated vasodilation. On the other hand, Todd *et al.* have shown that NOS inhibition reduces CBF proportionately in both isoflurane-anesthetized and barbiturate-anesthetized rabbits, indicating that NO is not the primary mediator of the isoflurane-induced vasodilation,

but acts to affect the background vasomotor tone.²⁰ In addition, isoflurane has been shown to attenuate endothelium-dependent, NO-mediated dilation EDD in normal cerebral arteries⁹ and this has been corroborated in this study.

Our study examines the complex relationship between isoflurane and endothelium-mediated vasomotion of the cerebral arterioles in a rat model of SAH. The limitations of our model include the fact that we are only studying the acute phase after SAH, whereas cerebral vasospasm in humans is biphasic with both an acute and a delayed phase.^{2,3} However, acute-phase vasospasm is predictive of mortality after SAH²¹ and may be no less important than delayed-phase vasospasm. Secondly, we examined vasomotion *in vitro* and did not measure CBF per sec. Our findings will have to be interpreted in association with studies on CBF. Thirdly, in this study, we did not include a sham-operated control group. In our previous study,⁵ where we did include a sham-operated group, we found that the effect of SAH on endothelial dysfunction could be demonstrated over and above that of a sham surgery and therefore, we did not feel that we had to include a sham-operated group for the present study.

Our main findings about the relationship between isoflurane and endothelium-mediated vasomotion in cerebral arterioles after SAH are twofold. First, we previously reported that there is impairment of EDD after SAH and suggested that this may be a factor predisposing the cerebral vessels to vasospasm.⁵ If isoflurane, which causes attenuation of EDD in normal cerebral arterioles, were to cause a similar effect in vessels with endothelial dysfunction after SAH, the anesthetic might lead to a profound impairment of EDD and an increased disposition to vasospasm. However, we actually found that isoflurane caused no further impairment of EDD in vessels after SAH; cerebral arterioles showed similar degrees of EDD after SAH in the presence or absence of isoflurane.

Analogously, impairment of EDD has been observed in various pathological situations in addition to SAH. It has been noted in coronary artery disease,²² hypercholesterolemia,²³ diabetes,²⁴ proteinuria,²⁵ and following cardiopulmonary bypass (CPB)⁹ - just to name a few. If isoflurane had an additive effect on impairment of EDD in these pathological conditions, then the anesthetic might contribute to end-organ ischemia in such situations. However, our studies in postCPB cerebral vessels⁹ and now in postSAH cerebral vessels demonstrate that there is no such additive effect. In humans after SAH, isoflurane has also been shown to maintain CBF.¹⁵

Previously, we found that, after SAH, there is an accentuation of ET-1 induced constriction of cerebral

arteries and this may be another factor contributing to vasospasm after SAH. We suggested that this accentuation might be related to reduced endothelial modulation of ET-1 induced vasoconstriction.⁵ Although EDD after SAH is not impaired further by isoflurane, the endothelium is still dysfunctional due to the effect of SAH and ET-1 induced vasoconstriction might be expected to remain accentuated even in the presence of isoflurane. However, isoflurane also has a direct, endothelium-independent cerebral vasodilatory effect²⁶⁻²⁸ and this latter effect may be expected to counterbalance the accentuated constriction to ET-1. Indeed we found that, in the presence of isoflurane, accentuation of ET-1 effect was reduced. Interestingly, Ringaert *et al.*²⁹ found that the increase in CBF produced by isoflurane is greater under hypocarbia when the background cerebral vessel tone is high than under hypercarbia when the tone is low. Similarly, attenuation of ET-1 induced vasoconstriction by isoflurane was greater in vessels after SAH than in control vessels, indicating that the cerebral vasodilatory effect of isoflurane depends on the pre-existing tone of the vessels.

In summary, using a rat model of the acute phase of SAH, we have found that the cortical microvessels have an attenuated EDD and accentuated response to ET-1 after SAH and that isoflurane does not further impair EDD, but modulates the accentuated response to ET-1 in these vessels. Such an effect of isoflurane would not predispose the vessels to vasospasm after SAH.

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