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The Dose-related Effects of Nitric Oxide Synthase Inhibition on Cerebral Blood Flow during Isoflurane and Pentobarbital Anesthesia

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Background: Recent work in animals suggests that nitric oxide may play a role in the cerebral blood flow (CBF) changes produced by anesthetics, particularly the vasodilation seen with volatile anesthetics. It is not clear, however, whether nitric oxide causes the flow increase or simply plays some constitutive role. To distinguish between these possibilities, we studied the dose-related effects of nitric oxide synthase inhibition in rabbits with varying baseline CBFs, produced by anesthesia with isoflurane, low-dose pentobarbital, or high-dose pentobarbital.

Methods: New Zealand White rabbits were anesthetized with 1 MAC isoflurane, low-dose pentobarbital (50-mg/kg load, 7.5-mg·kg^{-1}·h^{-1} infusion), or high-dose pentobarbital (50-mg/kg load, 20-mg·kg^{-1}·h^{-1}, deep burst-suppression on the electroencephalogram), and prepared for the measurement of CBF using radioactive microspheres. The confluence of sinuses was also exposed to permit sampling of cerebral venous blood and the determination of cerebral metabolic rate for O_2 (CMR_{O_2}). Normocapnia and normothermia were maintained throughout. After baseline measurements, animals were sequentially given a cumulative total of 3, 13, and 43 mg/kg intravenous N$\bar{\omega}$-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase. CBF and CMR_{O_2} were recorded ≈10 min after each dose.

Results: L-NAME produced a dose-related reduction in CBF in all three anesthetic groups. Statistical examination indicated that the dose response curves were parallel. For example, in isoflurane-anesthetized rabbits, CBF decreased from 77 ± 19 to 47 ± 11 ml·100 g^{-1}·min^{-1} after the 43 mg/kg L-NAME, whereas in high-dose pentobarbital animals, CBF decreased from 42 ± 15 to 26 ± 8 ml·100 g^{-1}·min^{-1} (all values mean ± SD). Decreases in CMR_{O_2} did not quite achieve significance (P = 0.08), and the CBF/CMR_{O_2} ratio decreased in all animals, suggesting that the CBF reductions were due primarily to direct vasoconstriction. There were no electroencephalographic changes. In separate groups of isoflurane-anesthetized rabbits given 3 mg/kg L-NAME, treatment with 300 mg/kg L-arginine partially reversed the decreases in CBF. By contrast, the effects of 43 mg/kg L-NAME were not reversible with 430 mg/kg L-arginine.

Conclusions: Although L-NAME reduced CBF in all three anesthetic conditions, it did not alter the relative differences among them: CBF in the presence of isoflurane remained much higher than that seen with the barbiturates. This suggests that nitric oxide may not the primary mediator of anesthetic CBF effects, but rather acts to influence background vascular tone in these anesthetized animals. (Key words: Anesthetics, intravenous: pentobarbital. Anesthetics, volatile: isoflurane. Arteries, cerebral: endothelium-derived relaxing factor. Brain: cerebral blood flow; cerebral metabolic rate. Anesthetics, gases: nitric oxide; nitric oxide synthase.)

BOTH volatile and intravenous anesthetics have profound effects on cerebral blood flow (CBF). However, the mechanisms by which these flow changes occur remain obscure. Changes in CBF produced by volatile anesthetics are determined by a combination of direct vasodilation,[1,2] alterations in cerebral metabolic rate and other unidentified mechanisms.[3–5] Barbiturates can also relax preconstricted, isolated cerebral arteries[6,7] but *in vivo* reduce CBF. This is probably an indirect effect, occurring secondary to a reduction in synaptic activity and cerebral metabolic rate.[8–10]

These findings imply that the observed CBF effects of anesthetics involve multiple interacting mechanisms. Recent studies using L-arginine analogs that inhibit nitric oxide synthase (NOS) have suggested that nitric oxide (NO) plays a role in controlling resting CBF,[11] autoregulation,[12] CO_2 responsiveness[13–15] and the coupling between CBF and cerebral metabolic rate.[16] More importantly, several groups of investigators have shown that NOS inhibition with N$\bar{\omega}$-nitro-L-arginine methyl ester (L-NAME) can reduce CBF during both isoflurane and halothane anesthesia[12,17] and can block the CBF increase produced by isoflurane.[18,19] In contrast, two

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other groups, working with barbiturate or chloralose-anesthetized animals (and with other NOS antagonists) failed to find changes in CBF.[20,21] These disparate observations suggest that the role of NO in determining the CBF responses to anesthetics is not a simple one. For example, it is possible that volatile anesthetics increase CBF by increasing the production of NO, whereas barbiturates reduce NO concentrations.

To examine further the role of NO in the anesthetic-induced CBF, we examined the response of CBF to NOS inhibition in anesthetized rabbits with widely differing baseline flows. This was done using either isoflurane or two different doses of pentobarbital. The results suggest that NO is unlikely to be the principal mediator of CBF changes during anesthesia, but instead provides a "tonic" background effect on the cerebral vasculature.

Materials and Methods

All procedures were approved by the University of Iowa Animal Care and Use Committee.

Male New Zealand White rabbits weighing 3.5–4.0 kg were initially placed in a closed plastic box and anesthetized with 5% halothane in O_2. A catheter was inserted into a marginal ear vein, a tracheostomy was performed, 1.25 mg/kg succinylcholine was given intravenously, and mechanical ventilation begun using O_2/N_2 (fraction of inspired O_2 = 0.40). Tidal volume was set at ≈35–40 ml and the ventilator rate was adjusted to insure normocarbia as initially assessed by expired gas analysis and later by arterial blood gas measurement. An intravenous infusion of normal saline solution was started at a rate of approximately 3–4 $ml \cdot kg^{-1} \cdot h^{-1}$. Succinylcholine was added to this infusion to ensure the administration of ≈2.5 $mg \cdot kg^{-1} \cdot h^{-1}$. An esophageal thermistor was placed and temperature was thereafter maintained at ≈38–39°C, using a heating pad.

Immediately after this initial preparation was complete, halothane was discontinued and animals were assigned to one of three experimental groups. In the first group ("isoflurane"), isoflurane was added to the inspired gas mixture and the end-expiratory concentration adjusted to 2.1–2.2% (1 MAC[22]), using a gas analyzer (Datex 254, Helsinki, Finland). This was continued for the remainder of the study. In these animals, phenylephrine was infused in doses sufficient to ensure that mean arterial pressure (MAP) remained at values at least 80 mmHg at all times. In the second group ("pentobarbital-low"), 50 mg/kg pentobarbital was given slowly *via* the ear vein catheter, followed by a continuous infusion of 7.5 $mg \cdot kg^{-1} \cdot h^{-1}$. In the third group ("pentobarbital-high"), 50 mg/kg pentobarbital was also given but was followed by a continuous infusion at the rate of 20 $mg \cdot kg^{-1} \cdot h^{-1}$. No vasopressors were needed in pentobarbital treated rabbits. Animals in all three groups were subsequently treated identically.

After group assignment, surgical preparation proceeded. Catheters were surgically placed into both femoral (PE 90) and both brachial arteries (PE 160) and into one femoral vein (PE 90). One of the femoral arterial catheters was constructed with a "pigtail" tip which was advanced into the left ventricular cavity, using the transduced pressure waveform as a guide. This catheter was used for subsequent microsphere injections. Each animal was then turned prone and the head fixed into a stereotactic frame, with the interaural line located approximately 10 cm above the table and sternum. A midline scalp incision was made and the confluence of venous sinuses was exposed *via* a small midline occipital craniectomy. Finally, all wounds were infiltrated with 0.25% bupivacaine, and two platinum needle electrodes were placed against the skull at the base of each ear. Continuously monitored variables in each animal now consisted of MAP, esophageal temperature, expired CO_2 and volatile agent concentrations and a single biparietal electroencephalogram (EEG). Arterial CO_2 tension was kept between 36 and 42 mmHg, while *p*H was maintained at greater than 7.30 by using sodium bicarbonate if needed.

Two hours after discontinuation of halothane (and ≈0.5–1 h after completion of surgery), arterial blood was drawn and MAP recorded. A sample of cerebral venous blood was obtained by puncturing the confluence of sinuses with a 25-G spinal needle mounted on a micromanipulator. O_2 tension, CO_2 tension, and *p*H were determined on both arterial and venous blood samples using a blood gas analyzer (IL 1306, Instrumentation Laboratories, Lexington, MA). In addition, total hemoglobin concentration, percentage oxyhemoglobin, and hemoglobin-bound O_2 content were measured on a Radiometer OM3 co-oximeter which was adjusted for use with rabbit blood. Total arterial and cerebral venous O_2 contents were calculated as the sum of hemoglobin-bound and dissolved O_2 (0.003 × O_2 tension). Finally, CBF was measured by the injection of 15 μm diameter radioactive microspheres (labeled with ^{153}Gd, ^{113}Sn, ^{85}Sr, or ^{46}Sc) into the left ventricle

via the pigtail catheter. Reference arterial samples were withdrawn simultaneously from both brachial arteries at a rate of 1 ml/min for 2 min, starting ≈15 s before microsphere injection.

After baseline measurements, each animal received an intravenous injection of 3 mg/kg L-NAME (cat. no. N5751, Sigma, St. Louis, MO) dissolved in normal saline. Ten minutes later, data were again collected and a second microsphere injection performed. Subsequent injections of 10 and 30 mg/kg L-NAME were then given (for cumulative doses of 13 and 43 mg/kg), with data obtained approximately 10 min after each injection.

When the experiment was complete, animals were killed with KCl. The brains were removed, and placed in formalin. Twenty-four hours later the fixed brain was divided into forebrain and hind brain portions at the level of the colliculi, and the forebrain was divided into left and right hemispheres (with underlying gray matter and portions of the rostral midbrain). The midbrain and brainstem caudal to the colliculi and the cerebellum were also isolated. The samples were weighed, and radioactivity was determined, along with the arterial reference samples, in a Packard Autogamma Counter. For each sample, CBF was calculated according to standard equations.[23,24] CBF values were used only when the counts in the two reference samples did not differ by more than 10% (to insure adequate microsphere mixing). Left and right hemispheric values were combined to yield "forebrain" CBF. The cerebral metabolic rate for O_2 (CMR_{O_2}) was calculated as arterial minus cerebral venous O_2 contents, multiplied by forebrain CBF. The ratio of forebrain CBF to CMR_{O_2} was also calculated, along with the O_2 extraction ratio (OER, arterial minus cerebral venous O_2 contents divided by arterial O_2 content).

Additional Experiments: L-Arginine Reversal

In an effort to verify the specificity of L-NAME for NOS, an attempt was made to reverse its effect by administering the normal substrate for NOS, L-arginine. Three rabbits were prepared as above, using isoflurane as the anesthetic; the confluence of venous sinuses was not exposed. After baseline measurements, L-NAME was given as above, *i.e.*, in cumulative doses of 3, 13, and 43 mg/kg. CBF was measured after each dose. After the final L-NAME dose was given, L-arginine (Sigma) 430 mg/kg was given intravenously, and flow was measured one more time.

The results of this experiment (see below) suggested that the 43 mg/kg L-NAME represented a very high and irreversible dose, at least with easily tolerated doses of L-arginine. Therefore, another five animals were anesthetized with isoflurane and prepared as above (*i.e.*, the confluence of sinuses was not exposed). After baseline measurements, 3 mg/kg L-NAME was given, followed by 30 and then 300 mg/kg L-arginine, with CBF determined after each dose.

Statistics

All results were compared between groups by using a two-way analysis of variance, with anesthetic group treated as an intergroup factor and L-NAME dose treated as a repeated, intragroup factor. For intergroup comparisons at a single L-NAME dose, a Fisher's LSD test was used, with significance accepted only for a P value of <0.02 (to minimize a the risk of a multiple comparison error). CBF/CMR_{O_2} ratios were log transformed before statistical examination.

Results

Data for the three groups are shown in table 1, and CBF changes are shown in figure 1. As expected, MAP was slightly greater in the low-dose pentobarbital group, and administration of L-NAME resulted in small increases in MAP in all groups. There were no significant intergroup differences or drug-related changes in arterial O_2 tension, arterial CO_2 tension, or pH. Arterial hemoglobin was 12.6 ± 0.9 g/dl under baseline conditions (all groups combined) and fell slightly over the course of the experiment to 12.3 ± 0.9 g/dl. Hematocrit did not change significantly (not shown). There were no intergroup differences.

There were no regional differences in the response to L-NAME administration and hence only forebrain values are reported. As expected, baseline CBF was greatest for isoflurane-anesthetized rabbits, and lowest for animals receiving high-dose pentobarbital (figure 1). In each group, L-NAME administration produced a dose-related reduction in CBF. However, in spite of the differing baseline CBF values observed with the three agents, two-factor analysis of variance indicated that the three L-NAME dose–response curves were parallel (P value for the A × B interaction was 0.13). Stepwise analysis of variance after excluding each lower L-NAME dose (*e.g.*, 3 *vs.* 13 and 43 mg/kg; 13 *vs.* 43 mg/kg) showed that each added dose produced another incremental (although smaller) fall in CBF. However, for no dosage range did the A

Table 1. Experimental Variables

	Baseline	L-NAME Dose		
		3 mg/kg	13 mg/kg	43 mg/kg
Mean arterial pressure (mmHg)				
Isoflurane (n = 8)	93 ± 10	94 ± 4	95 ± 5	95 ± 5
Pentobarbital, low (n = 8)	108 ± 10*	115 ± 12*	116 ± 10*	116 ± 10*
Pentobarbital, high (n = 8)	94 ± 11	99 ± 12	98 ± 12	93 ± 14
Pa_{O_2} (mmHg)				
Isoflurane	138 ± 12	142 ± 10	146 ± 11	146 ± 6
Pentobarbital, low	145 ± 8	148 ± 10	148 ± 8	152 ± 4
Pentobarbital, high	157 ± 13	154 ± 14	156 ± 12	157 ± 11
Pa_{CO_2} (mmHg)				
Isoflurane	37 ± 2	38 ± 2	37 ± 2	39 ± 2
Pentobarbital, low	37 ± 2	36 ± 1	37 ± 1	37 ± 1
Pentobarbital, high	37 ± 2	38 ± 4	38 ± 3	38 ± 2
*p*H (units)				
Isoflurane	7.42 ± 0.04	7.38 ± 0.06	7.37 ± 0.06	7.37 ± 0.05
Pentobarbital, low	7.37 ± 0.05	7.38 ± 0.04	7.35 ± 0.04	7.35 ± 0.05
Pentobarbital, high	7.40 ± 0.05	7.39 ± 0.06	7.38 ± 0.04	7.37 ± 0.02
Hgb (g/dl)				
Isoflurane	12.4 ± 0.9	12.5 ± 0.8	12.5 ± 1.0	12.5 ± 1.2
Pentobarbital, low	12.9 ± 1.0	12.4 ± 1.0	12.5 ± 1.1	12.4 ± 0.8
Pentobarbital, high	12.4 ± 0.7	12.6 ± 0.7	12.1 ± 0.8	12.0 ± 0.7
fCBF ($ml \cdot 100\ g^{-1} \cdot min^{-1}$)				
Isoflurane	77 ± 19	53 ± 11	49 ± 10	47 ± 11
Pentobarbital, low	62 ± 20	49 ± 14	44 ± 8	42 ± 8
Pentobarbital, high	42 ± 15*	32 ± 13*	29 ± 10*	26 ± 8*
$CMRO_2$ ($ml \cdot 100\ g^{-1} \cdot min^{-1}$)				
Isoflurane (n = 6)	3.26 ± 1.03	3.32 ± 0.43	3.27 ± 0.34	2.99 ± 0.54
Pentobarbital, low (n = 7)	3.43 ± 0.66	3.88 ± 0.86	3.67 ± 0.70	3.40 ± 0.42
Pentobarbital, high (n = 7)	2.76 ± 0.82	2.41 ± 0.76*	2.25 ± 0.59*	2.16 ± 0.42*
$CBF/CMRO_2$ ratio				
Isoflurane	24.3 ± 8.0	16.6 ± 2.0	15.4 ± 3.4	16.2 ± 2.2
Pentobarbital, low	19.2 ± 9.4	12.7 ± 2.4	12.2 ± 2.3	12.3 ± 2.3
Pentobarbital, high	15.7 ± 5.1	14.0 ± 6.0	12.8 ± 3.7	12.4 ± 3.8
OER				
Isoflurane	0.26 ± 0.07	0.36 ± 0.05	0.40 ± 0.08	0.38 ± 0.07
Pentobarbital, low	0.35 ± 0.14	0.47 ± 0.08	0.50 ± 0.08	0.50 ± 0.08
Pentobarbital, high	0.40 ± 0.15	0.46 ± 0.13	0.49 ± 0.12	0.51 ± 0.13

Values are mean ± SD.

fCBF: forebrain cerebral blood flow; $CMRO_2$: cerebral metabolic rate for O_2; OER: oxygen extraction ratio.

* Value is significantly different from the other two groups at a $P < 0.01$ (Fisher's LSD test). Statistics regarding repeated measurements can be found in the text.

× B interaction become significant, again suggesting parallel responses.

Inability to obtain venous blood samples at all 4 measurement points reduced the number of animals in which CMR_{O_2}, CBF/CMR_{O_2} ratios, and O_2 extraction ratio could be calculated to 6, 7, and 7 in the isoflurane, pentobarbital-low, and pentobarbital-high groups, respectively. Although baseline CMR_{O_2} differed among groups, two-factor analysis of variance indicated that the small decrease in CMR_{O_2} observed after L-NAME did not achieve significance ($P = 0.08$). There was no significant A × B interaction ($P = 0.48$). The direct vasoconstrictive effects of L-NAME were evidenced by the significant (but again parallel) decreases in the CBF/CMR_{O_2} ratio. O_2 extraction ratio rose significantly in all three groups, but with no intergroup differences.

Animals in both the isoflurane and pentobarbital-low groups showed continuous activity on the EEG, largely composed of a mixture of predominantly high amplitude delta and theta activity, with superimposed low

amplitude, higher frequency waves (usually in the alpha range). By contrast, animals in the pentobarbital-high groups showed deep burst suppression, often with interburst intervals of 10–30 s. Despite these baseline differences, visual inspection of the EEG recordings failed to reveal any consistent changes in any group during L-NAME administration.

In the 3 supplementary isoflurane-anesthetized animals given 43 mg/kg L-NAME, forebrain CBF decreased from 87 ± 26 ml · 100 g^{-1} · min^{-1}, to 36 ± 7 ml · 100 g^{-1} · min^{-1}. The administration of L-arginine had no influence on CBF (CBF after L-arginine 38 ± 10 ml · 100 g^{-1} · min^{-1}). In the second group of five animals, 3 mg/kg L-NAME reduced CBF from 101 ± 47 to 66 ± 25 ml · 100 g^{-1} · min^{-1}. L-arginine 30 mg/kg did not change CBF (70 ± 47 ml · 100 g^{-1} · min^{-1}), but 300 mg/kg L-arginine increased CBF to 80 ± 31 ml · 100 g^{-1} · min^{-1} ($P = 0.02$ *vs.* post-L-NAME).

Discussion

Although some contradictory results have been presented,[20,21,25] most laboratories have shown that L-NAME and other NOS inhibitors decrease CBF or constrict the cerebral vasculature, or do both, in both awake and anesthetized animals.[11,13,15,17,26–29] These findings support the widely accepted concept that NO plays a role in cerebrovascular control. However, they do not resolve the issue of whether NO plays some role in the specific cerebrovascular changes produced by anesthetics. For example, it is not clear whether the increase in CBF produced by agents such as halothane or isoflurane is caused by increases in NO (or perhaps and increased sensitivity to NO) or whether the reduction in CBF produced by drugs such as the barbiturates involves reductions in NO action.

There are two general approaches to this question. In the first of these, Moore *et al.*[18] and McPherson *et al.*[19] demonstrated that the treatment of dogs with L-NAME markedly attenuated the expected increase in CBF produced by isoflurane. In a similar fashion, Koenig *et al.* showed that L-NAME administration blocked the usual dilation of pial vessels in response to halothane (although the response to nitroprusside, a direct nitrovasodilator, was not altered).[30] These experiments would appear to suggest that NO was a specific component of the vasodilatory action of these vasodilators.

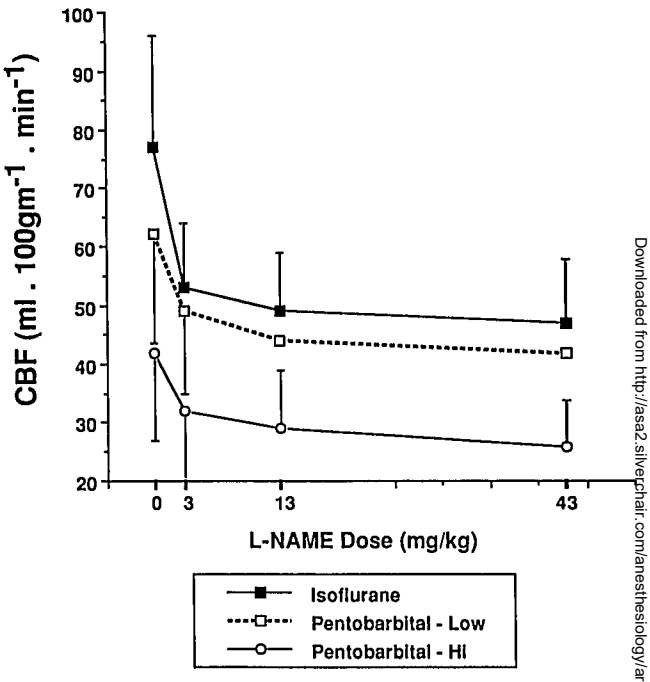

Fig. 1. Forebrain cerebral blood flow (CBF) in the three anesthetic groups is plotted against the three Nω-nitro-L-arginine methyl ester (L-NAME) dose levels (presented as cumulative dose). Points are mean ± SD; for clarity, not all SD bars are shown.

However, Wei *et al.* examined the response to L-NAME in both awake and isoflurane anesthetized rats and found that the drug produced very similar fixed increases in vascular resistance in both groups, even though pretreatment CBF was much greater with isoflurane.[17] They concluded that basal activity of NOS plays a role in the control of vascular tone, but there was no unique difference between awake and anesthetized rats.

Our experiment was performed in a fashion similar to that of Wei *et al.*[17] We chose three anesthetic conditions with widely differing CBF and CMR_{O_2} conditions. Based on the work of Wang *et al.* with halothane and nitro-L-arginine[13] and our own observed reductions in cortical perfusion (using the laser Doppler) in halothane-anesthetized rats given L-NAME,‖ we expected to see a large L-NAME-induced CBF reduction in isoflurane anesthetized animals. We expected to see little or no CBF reduction in animals whose cerebral vasculature was vasoconstricted by pentobarbital (where little NO mediated anesthetic-induced "vasodilation" would be expected). In other words, if NO were the principal mediator of the increased CBF with isoflurane, we

‖ Verhaegen M: Unpublished data.

should have seen convergence of the L-NAME/CBF dose–response curves for the three anesthetic states. Instead, we observed essentially parallel dose response curves for the three groups, and found that the proportional differences in CBF which existed among the three anesthetics under baseline conditions were still present after the maximal dose of L-NAME. For example, under baseline conditions, CBF with isoflurane was 183% of that seen with high-dose pentobarbital. After the largest dose of L-NAME, isoflurane CBF was still 181% of that seen with pentobarbital. This parallel response to NOS inhibition has a number of implications. Since L-NAME failed to abolish the *differences* between the CBF effects of the three anesthetics, it is reasonable to conclude that the differing effects of these drugs on CBF is independent of NO: even after near-maximal inhibition of NOS (and presumably with little available NO in the tissue), isoflurane still results in greater CBF than does pentobarbital. This implies that although NO plays a role in the control of CBF, it cannot be the direct mediator of isoflurane-induced cerebral vasodilation. A more reasonable hypothesis is that some background NO-mediated vasodilation was present in all three anesthetic groups. Comparable degrees of NOS inhibition (which should produce comparable reductions in NO) resulted in parallel CBF decreases.

There is, of course, an alternative hypothesis. It is possible that all 3 of the anesthetic regimens (or perhaps anesthesia *per se*) produce an equivalent degree of NO-mediated vasodilation under baseline conditions. This hypothesis requires the unusual step of ascribing "vasodilatory" effects to barbiturate anesthesia. It is generally believed that most of the *in vivo* CBF effects of barbiturates are indirect, occurring as a coupled response to the reduction in cerebral metabolic activity.[8–10,31–33] This is evidenced by the unchanging CBF/CMR_{O_2} ratio seen during barbiturate loading. Both *in vitro* and *in vivo* studies suggest that barbiturates do indeed possess some cerebrovasodilatory effects. Work with preconstricted, isolated cerebral vessels indicates that both pentobarbital and thiopental can produce vessel relaxation.[6,7,34] In addition, Messeter *et al.* noted that whereas thiopental reduced CBF in head-injured patients with normal vascular responses to changing arterial CO_2 tension, it increased CBF in more severely injured patients in whom CO_2 response was absent, and in whom either baseline CMR_{O_2} was already minimal or CBF/CMR_{O_2} coupling was probably absent.[35] Added support for this can be found in the exaggerated systemic hypertension seen in barbiturate-anesthetized rats given NOS inhibitors, an observation that suggests barbiturate-enhanced NO activity.[36,37] However, both halothane and enflurane appear to attenuate this pressor response, suggesting again that no consistent relationship between NO activity and anesthetics is present.[37]

The concept that NO or endothelium-derived relaxing factor is the cause of anesthetic-induced cerebral vasodilation is also at odds with available *in vitro* studies of isolated cerebral and extracranial vessels. Initial studies with isolated vessels suggested that removal of the endothelium does not alter isoflurane-induced relaxation in cerebral arteries,[1,2] and studies on rat aorta indicate that volatile anesthetics may actually inhibit endothelium-dependent relaxation.[38] In addition, although volatile anesthetics increase total cyclic guanosine monophosphate in vascular smooth muscle, they do not increase the concentration of the soluble cyclic guanosine monophosphate fraction (which is modulated by NO).[39] More recent studies suggest that halothane may actually attenuate the action of NO in isolated vessels.[40] In fact, Tobin *et al.* have recently shown that halothane inhibits NOS activity in vitro.[41] Furthermore, in the aforementioned studies by Koenig *et al.*[30] and by McPherson *et al.*,[19] L-NAME treatment did not completely block anesthetic-mediated vasodilation, but only attenuated it. Perhaps in a similar fashion, NOS inhibition attenuates—but does not obliterate—the CBF response to CO_2.[13–15] This incomplete effect of NOS blockade suggests that NO plays a role in both anesthetic and CO_2 induced vasodilation, but that it cannot be the sole mediator. Other autocoids may play an important role. For example, Moore *et al.* have provided data suggesting some interaction between NO and prostanoids in determining the response to isoflurane,[18] and cycloxygenase inhibition with indomethacin may reduce CBF and attenuate CO_2 responsiveness.[42,43]

Some aspects of our study design requires some comment. We chose to examine the three anesthetic states because it was possible to achieve widely different flow conditions in which to test NOS inhibition and to compare an anesthetic that is considered an *in vivo* "vasodilator" (isoflurane) with a "vasoconstrictor" agent (pentobarbital). Obviously, other conditions could have been chosen (*e.g.*, different volatile agents or different doses of volatile agents *vs.* barbiturates or fentanyl). NOS inhibition reduces CBF in animals given different anesthetics, but it is possible that the shape of the CBF dose–response curves might have differed. It is also possible that very different

results might have been found if other anesthetics had been tested, although the only other comparison of two different anesthetic states (isoflurane *vs.* awake) demonstrated results qualitatively similar to our own.[17] Although we did not include time-control groups in our study, preliminary work in our laboratory indicated that CBF was stable in both isoflurane and pentobarbital-anesthetized rabbits over a period approximately equivalent to the period of L-NAME administration used here. Therefore, the changes seen in our study cannot be due to time-linked effects. Another aspect of our results was somewhat surprising, and that is the minimal MAP changes in rabbits given L-NAME, a finding that differs dramatically from that seen in rats where often severe hypertension is the rule.[44] This is not surprising in the isoflurane-anesthetized animals since a phenylephrine infusion was adjusted to maintain MAP, and since volatile anesthetics attenuate the response to NOS inhibitors, at least in rats.[37] However, the lack of a response in pentobarbital-anesthetized rabbits was more surprising. Several explanations are possible. First, the rabbit may be less sensitive to NOS inhibition induced hypertension. Both Rees *et al.*[45] and Faraci and Heistad[21] noted that the MAP increases produced by L-NMMA or L-NNA respectively appeared to be less than seen in rats. In addition, although heart rate was not monitored in our primary study, we did see a marked slowing of heart rate in later pentobarbital-anesthetized animals given L-NAME, suggesting that baroreflexes may play an important role. However, a similar heart rate slowing was not seen with isoflurane.

One issue that was not addressed completely is the possibility that L-NAME may have effects other than NOS inhibition. As noted in results, we were unable to reverse the effects of our highest L-NAME doses with L-arginine, although partial reversal of the lowest dose was possible. This may be related to the relative affinity of NOS for L-NAME and L-arginine, or perhaps the inhibition of L-arginine uptake (although L-NAME does not appear to inhibit L-arginine transport in cultured endothelial cells[46]). It is also possible some NOS-independent effect of L-NAME was present. For example, L-NAME has anticholinergic actions in the peripheral vasculature,[47] and the cholinergeric nervous system is well known to play a role in cerebral vasomotor control, particular vasodilation.[48] At present, this possibility cannot be eliminated.

We also measured CMR_{O_2} in our animals, primarily to rule out the possibility that major flow changes produced by L-NAME might be indirectly due to metabolic depression. The experiment failed to show any significant change in CMR_{O_2}, although power estimates suggest that if group sizes had been doubled, significance might have been achieved. This small change in CMR_{O_2} is consistent with the work of Iwamoto *et al.*[25] However, it also seems surprising in view of the finding by Johns *et al.*[49] that L-NAME doses similar to those used here reduced halothane MAC by almost 50%, and the well known role of NO in excitatory NMDA receptor/glutamate-mediated neuronal systems.[50–52] We did not observe any marked effects of L-NAME loading on the EEG in any animal. This latter finding differs from the work of Kovach *et al.*, who noted significant reductions in total EEG power in chloralose/urethane-anesthetized cats.[53]

These latter observations concerning the influence (or lack of influence) of L-NAME on CMR_{O_2} and on the EEG raises the issue of NO as a neurotransmitter. Most discussion of the effects of NO or NOS inhibition on CBF seem to consider the changes as primarily due to endothelial processes. Endothelium-derived NO is unquestionably key to the flow changes seen with NOS inhibition in some organs, but it may not be true in brain.[54] As noted, *in vitro* studies with volatile agents and cerebral vessels have not consistently shown endothelial dependence. It is hence possible that neuronally generated NO is more important. Given the tight coupling between neuronal activity, cerebral metabolism and CBF, it would not be surprising if neurons (or their adjacent glia) were capable of elaborating vasoactive compounds.[55] It is also possible that the small decreases in CMR_{O_2} reflects a reduction in the activity of a select neuronal population which might contribute to the decreases in CBF which were observed. However, since synaptic activity was already near-maximally suppressed in the high dose pentobarbital group, this CMR_{O_2} effect of L-NAME may not be linked to either the anesthetic effects of the drug, or with synaptic activity as evidenced by the EEG.

In summary, we observed a dose-related reduction in CBF in rabbits treated with a competitive NOS inhibitor. This was true regardless of whether the animals were anesthetized with isoflurane or either low or high dose pentobarbital. The apparently parallel character of the L-NAME dose–response curves, and the fact that NOS inhibition did not obliterate the differences among these three anesthetic conditions, suggests that although NO plays an important role in overall CBF control and is probably a constitutive part of the cerebral

milieu, it is not the primary mediator of the action of the vascular effects of either isoflurane or pentobarbital. NO may act as only one of many factors which define the cerebrovascular effects of anesthetics.

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