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Nitric Oxide and Prostanoids Contribute to Isoflurane-induced Cerebral Hyperemia in Pigs

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Background: **The mechanism of isoflurane-induced cerebral hyperemia is poorly understood. Data from studies *in vitro* suggest that volatile anesthetics release a vasodilator prostanoid. We hypothesized that prostanoids and nitric oxide (NO) are mediators of this response *in vivo*. If true, inhibition of cyclooxygenase by indomethacin (5 mg/kg intravenously) or of nitric oxide synthase by N^{ω}-nitro-L-arginine methyl ester (L-NAME; 40 mg/kg intravenously) should attenuate isoflurane-induced hyperemia. Any response to L-NAME occurring *via* nitric oxide should be competitively reversed by L-arginine.**

Methods: **The cerebral blood flow (microsphere) response to 1 MAC isoflurane was tested at three time points (0, 90, and 180 min) in pentobarbital-anesthetized pigs. Isoflurane challenges were separated by 60-min periods of continuous intravenous pentobarbital alone. Control animals (n = 7) received no additional pharmacologic intervention. Experimental animals were randomized to receive L-NAME before the second and indomethacin before the third isoflurane challenge (n = 7); L-NAME before the second and L-arginine (400 mg/kg intravenously) before the third isoflurane challenge (n = 9); or indomethacin before the second and L-NAME before the third isoflurane challenge (n = 8).**

Results: **In control animals, isoflurane reproducibly increased cerebral blood flow (whole brain; 113 ± 18%, 120 ± 18%, and 103 ± 19% increase above baseline at each time point, respectively). Both indomethacin and L-NAME attenuated (10 ± 10% and 52 ± 11% increase, respectively) the hyperemic response to isoflurane. The effect of L-NAME was reversed by L-arginine.**

Conclusions: **We conclude that both prostanoids and nitric oxide contribute to isoflurane-induced hyperemia. We are unable to determine from our data what, if any, interaction exists between these two mechanisms. (Key words: Anesthetics, gases: nitric oxide. Anesthetics, volatile: isoflurane. Brain: cerebral blood flow; cerebral metabolic rate of oxygen consumption. Inhibitors, cyclooxygenase: indomethacin. Vasodilation: endothelium-dependent. Vasodilators: prostanoids.)**

CEREBRAL hyperemia during inhalational anesthesia is recognized in several species,[1,2] but the mechanism of this hyperemia is poorly understood. Several theories involving the modulation of intercellular communication have been proposed, including increases in arachidonic acid metabolites,[3] and, more recently, production of nitric oxide (NO) or an NO-containing compound.[4] The role of prostanoids in isoflurane-induced hyperemia *in vivo* has not been studied. However, prostanoids *in vitro* appear to be involved in aortic vasodilation produced by volatile anesthetics.[3] Prostanoids are important mediators of cerebrovascular tone *in vivo* in a variety of neonatal porcine experimental conditions. For example, indomethacin, a nonspecific cyclooxygenase inhibitor, is known to modulate the cerebral vascular effects of hypercapnia and hypocapnia,[5] hypotension,[6] and asphyxia[7] in piglets. There is evidence that prostanoids may also modulate cerebrovascular reactivity in adults of other species.[8,9]

With regard to a mechanism involving NO, data from studies *in vitro* suggest that volatile anesthetics impair endothelium-mediated vasodilation[10] and that isoflurane-induced vasodilation cannot be prevented by inhibiting production of NO.[11,12] These studies, however, may not predict the effect of isoflurane on cerebral blood flow (CBF) *in vivo*. For example, NO is an important mediator of cerebral vasodilation produced by perivascular nerves,[13] and it may be produced in large quantities by NO synthase, which is present in astrocytes[14] and neurons.[15] Any contribution of these neural elements to cerebrovascular tone is excluded from an *in vitro* vessel preparation.

The purpose of this study was to determine if NO or a vasodilator prostanoid are important mediators of

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isoflurane-induced cerebral hyperemia *in vivo*. We tested the hypothesis that inhibition of NO or prostanoid synthesis would attenuate isoflurane-induced hyperemia. N^{ω}-nitro-L-arginine methyl ester (L-NAME) was administered as a prolonged competitive inhibitor of NO synthase,[16] and indomethacin was administered as an inhibitor of the cyclooxygenase pathway.[17] L-Arginine, the substrate from which NO is produced by NO synthase, was administered to reverse the effects of L-NAME.[18]

Materials and Methods

General Preparation

After the experimental protocol had been approved by the Johns Hopkins Animal Care and Use Committee, mixed-breed 8–10-week-old pigs (15–20 kg) of either sex were studied. The animals were allowed free access to food and water until the day of surgery to avoid dehydration and hypoglycemia. Anesthesia was induced with sodium pentobarbital (65 mg/kg intraperitoneally) and maintained with a continuous intravenous (iv) pentobarbital infusion at 5 $mg \cdot kg^{-1} \cdot h^{-1}$ for the remainder of the surgical preparation and experimental protocol. Tracheostomy was performed immediately after induction of anesthesia, and the lungs were mechanically ventilated (respirator model 607, Harvard). Tidal volume was set at 10 ml/kg, and the rate was adjusted to maintain the arterial partial pressure of carbon dioxide at approximately 40 mmHg. Supplemental oxygen was added to the inspired gas mixture to maintain the arterial partial pressure of oxygen greater than 100 mmHg. After adequate anesthesia (no purposeful movement or hypertension in response to surgical stimulation) had been achieved, the pig was paralyzed (with pancuronium bromide 1 mg/kg intramuscularly) to facilitate the use of electrocautery and to maintain a constant minute ventilation.

Catheters were placed in femoral and cephalic veins for administration of fluids and drugs. An axillary arterial catheter was inserted for the monitoring of mean arterial blood pressure (MABP) and arterial blood gases. A femoral arterial catheter was advanced proximally to the level of the midthorax and was used to obtain the reference sample during microsphere injection. A balloon-tipped catheter (10-ml balloon) was placed in the opposite femoral artery and advanced proximally 25–30 cm to the level of the low thorax. A second balloon-tipped catheter was placed in a femoral vein and advanced proximally in the vena cava to the same level. These balloon-tipped catheters were inflated as necessary to control MABP later in the protocol. A left thoracotomy was performed and a left atrial catheter placed for microsphere injection.

The animal was then turned prone, and the head was stabilized, with the external auditory meatus approximately 5 cm above the level of the heart. The superior sagittal sinus was exposed at the level of the coronal suture and cannulated (approximately 3–4 mm) in the direction of the confluence of the sinuses. This catheter was used to obtain cerebral venous blood samples for oxygen content analysis. A 2.5-mm silicone elastomer ventricular drain (Cordis, Miami, FL) was inserted into the right lateral ventricle to monitor intracranial pressure (ICP). A thermistor (Mon-A-Therm, LaBarge, St. Louis, MO) was placed into the parenchyma of the right parietal lobe to monitor brain temperature. Brain temperature was maintained at 38.0 ± 0.5°C with a heat lamp and heating pad.

MABP and ICP were measured continuously with pressure transducers (P-23 ID, Statham, Oxnard, CA) and recorded with a polygraph (Gould-Brush, Cleveland, OH). Cerebral perfusion pressure was calculated as MABP − ICP. End-tidal carbon dioxide and isoflurane were measured continuously with an expired-gas analyzer (BP1000, Nellcor, Hayward, CA). Arterial oxygen and carbon dioxide partial pressures and *p*H were measured with electrodes (BMS3, Radiometer, Copenhagen, Denmark) and an analyzer (ABL3, Radiometer). Arterial glucose concentration was measured with a glucose analyzer (2300, Yellow Springs Instruments, Yellow Springs, OH). Arterial and cerebral venous oxygen contents, saturation, and hemoglobin concentration were determined with a Hemoximeter (OSM3, Radiometer, Copenhagen, Denmark). The blood gas analyzer and CO-oximeter were calibrated with air and mixtures of oxygen in nitrogen (8%) and carbon dioxide in air (5 and 10% carbon dioxide) to a precision of 0.1%. The *p*H electrode was calibrated with standard phosphate buffers (6.840 and 7.381).

Cerebral Blood Flow Measurements

Regional CBF was measured with reference sample radiolabeled microspheres.[19] Blood flow analysis was performed as previously described.[20] Briefly, microspheres 16 ± 0.5 μm in diameter (DuPont–New England Nuclear Products, Boston, MA) were injected into the left atrium and a reference sample withdrawn from the femoral artery catheter (tip confirmed above

the aortic balloon). The six isotopes (^{153}Gd, ^{114m}In, ^{113}Sn, ^{103}Ru, ^{95}Nb, and ^{46}Sc) used in each pig were injected in random sequence. The number of microspheres injected was chosen to allow at least 400 microspheres to be delivered to the smallest tissue sample taken. At the conclusion of the experiment, the pig was killed with iv potassium chloride and the brain removed for analysis.

After formalin fixation, the brain was sectioned to determine regional CBF to the following areas: hindbrain (brainstem and cerebellum) and forebrain (all brain except hindbrain). CBF is expressed in milliliters per minute per 100 g brain tissue by normalizing for tissue sample weights. Cerebral metabolic rate of oxygen consumption (CMR_{O_2}) was calculated (as forebrain blood flow × [arterial − venous oxygen content]) for each set of experimental conditions for each animal.

Protocol

After surgical preparation, animals were randomly divided into four groups (table 1). The goal MABP proximal to the aortic balloon was 110–120 mmHg for all groups; it was maintained throughout each protocol by inflating the aortic balloon to counteract the hypotensive effects of isoflurane or by inflating the vena caval balloon to counteract the hypertensive effects of indomethacin or L-NAME. Sodium bicarbonate was infused iv as needed (approximately 0.25–0.5 $mEq \cdot kg^{-1} \cdot h^{-1}$ of 1 mEq/ml sodium bicarbonate solution) to maintain physiologic *p*H in the setting of aortic or vena caval occlusion. Twenty minutes after completion of surgery, baseline measurement of CBF was made and all physiologic parameters recorded.

All animals received three transient challenges of 1 MAC isoflurane (1.4 vol%) at 0, 90, and 180 min. CBF was measured immediately before each isoflurane challenge and after equilibration with isoflurane, for a total of six measurements. We chose to use 1 MAC isoflurane because of difficulty, with greater concentrations, in maintaining a constant MABP during a basal pentobarbital anesthetic. This concentration of isoflurane produces cerebral hyperemia in other species.[21] During each isoflurane challenge, equilibration with 1 MAC isoflurane was allowed (approximately 15 min) and then this concentration maintained for 15 min before CBF was tested. Immediately after measurement of CBF during isoflurane, the agent was discontinued and the animals allowed to rest for 60 min while receiving pentobarbital alone, before the next baseline CBF test.

Table 1. Experimental Groups

Group	(n)	Drug Administered before Time (min) 0	90	180
Controls	(7)	No drug	No drug	No drug
Indo/L-NAME	(8)	No drug	Indo	L-NAME
L-NAME/Indo	(7)	No drug	L-NAME	Indo
L-NAME/L-ARG	(9)	No drug	L-NAME	L-ARG

Indo = indomethacin (5 mg/kg intravenous); L-NAME = N^{ω}-nitro-L-arginine (40 mg/kg intravenous); L-ARG = L-arginine (400 mg/kg intravenous).

Using a tissue–gas partition coefficient for whole brain of 3.65,[22] it can be predicted that 99% of the isoflurane should be cleared from brain within 30 min, assuming a constant CBF of 40 $ml \cdot min^{-1} \cdot 100\ g^{-1}$. Because of the time required to equilibrate at 1 MAC isoflurane, the protocol was designed for measurement of baseline CBF (during pentobarbital alone) every 90 min. For simplicity, both the baseline and the isoflurane CBF measurements are referred to as times 0, 90, or 180 min, according to whether they were associated with the first, second, or third isoflurane challenge, respectively. The response to isoflurane is described as the percent increase in CBF ($[(CBF_{isoflurane} - CBF_{baseline})/CBF_{baseline}] \times 100$). In control animals (n = 7) the CBF response to isoflurane was measured at these three time points (0, 90, and 180 min) without any other interventions. The goal of this control group was demonstration of consistent hyperemia with repetitive challenges of 1 MAC isoflurane.

In the indomethacin/L-NAME group (n = 8), the baseline CBF response to 1 MAC isoflurane was tested and then the animals allowed to rest for 60 min after discontinuation of isoflurane. At the second time point (90 min), the CBF response to isoflurane was retested 15 min after completion of an infusion of indomethacin (5 mg/kg with 60 mg sodium carbonate in 25 ml distilled deionized water iv over 15 min). This dose of indomethacin was chosen because it has been demonstrated to cross the blood–brain barrier and to reduce prostanoid concentration markedly in the subarachnoid space of piglets.[17] Animals were again allowed to rest for 60 min after discontinuation of isoflurane. At the third time point (180 min), the CBF response to isoflurane was retested 15 min after completing an infusion of L-NAME (40 mg/kg in 10 ml 0.9% saline iv over 15 min). L-NAME/indomethacin animals (n = 7) were subjected to the same experimental protocol as indo-

methacin/L-NAME animals, except that the order of drug administration was reversed.

In the fourth experimental group (L-NAME/L-arginine, n = 9), the baseline CBF response to 1 MAC isoflurane was tested as in other groups. At the second time point (90 min), the CBF response to isoflurane was retested 15 min after completion of an infusion of L-NAME (40 mg/kg). At the third time point (180 min), the CBF response to isoflurane was retested 15 min after completion of an infusion of L-arginine (400 mg/kg iv dissolved in 25 ml distilled deionized water over 15 min). Animals were allowed to rest for 60 min after each isoflurane challenge before further experimentation. In all experimental groups, the purpose of the 90-min data (second isoflurane challenge) was to evaluate independently the NO and prostanoid systems as potential mechanisms for isoflurane-induced hyperemia. The purposes of the 180-min data (third isoflurane challenge) were to see if the combination of NO synthase and prostanoid inhibition could further attenuate isoflurane-induced hyperemia (L-NAME/indomethacin and indomethacin/L-NAME groups) and to attempt to reverse the inhibitory effects of L-NAME on NO synthase by using L-arginine (L-NAME/L-arginine group).

Nitric Oxide Synthase Activity Assay

To determine the amount of NO synthase activity inhibition achieved in this age pig with L-NAME 40 mg/kg iv, an assay measuring NO synthase activity *in vitro* was performed on cortical biopsy samples removed sequentially from four pentobarbital-anesthetized pigs. After small burr holes had been made in the parietal cranium, biopsy samples were taken at baseline and then at 30, 75 (n = 4), and 140 min (n = 1) after completion of L-NAME 40 mg/kg iv. Using modifications of a previously described technique[23] to measure the conversion of [^{14}C]arginine to [^{14}C]citrulline by NO synthase, we found in these preliminary tests that L-NAME 40 mg/kg produced a 73 ± 5% reduction in NO synthase activity in cortex within 15 min of administration (n = 4). This inhibition of activity was sustained over the duration of the experiment (fig. 1).

Data Analysis

Each variable is expressed as the mean ± standard error. Four-way analysis of variance with three within-group factors (region, isoflurane challenge number, and isoflurane response) and one between-group factor (treatment group) was used for analysis of CBF. This analysis demonstrated statistical significance for all four factors. Therefore, to understand effects within each of the regions (forebrain and hindbrain) better, we used a three-way analysis of variance with two within-group factors (isoflurane challenge number and isoflurane response) and one between-group factor (treatment group). Final analysis of these data involved a two-way analysis of variance with two within-group variables (isoflurane challenge number and isoflurane response) for each group and for each region. The Newman–Keul test was used for *post hoc* comparisons. Two-way analysis of variance with repeated measures was used to test the effect of treatment on physiologic variables. Statistical significance was assumed when $P \leq 0.05$.

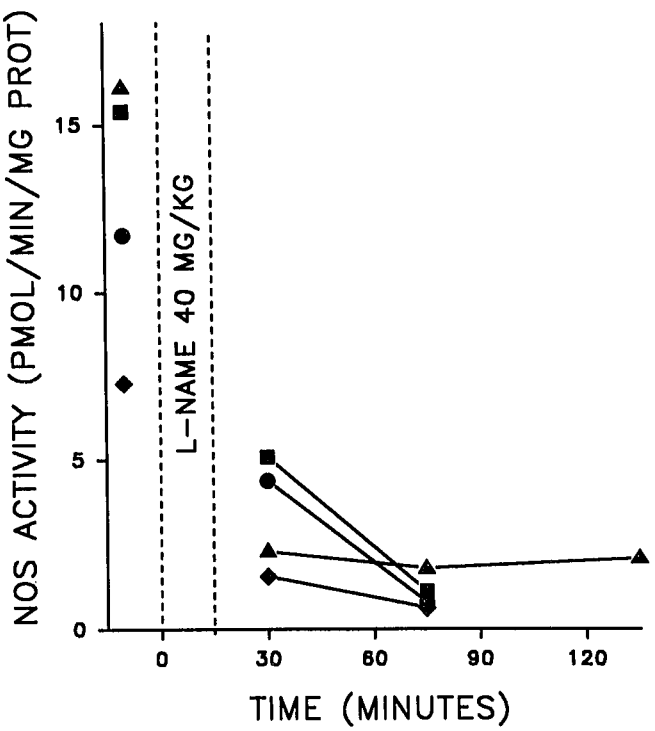

Fig. 1. Nitric oxide synthase (NOS) activity (picomoles per minutes per milligram protein) of cortical biopsy samples. N$^{\omega}$-nitro-L-arginine methyl ester (L-NAME) 40 mg/kg iv was administered over 15 min starting at time 0. Each set of symbols represents one pentobarbital-anesthetized pig (15–20 kg, n = 4); points to the left of time 0 represent baseline NOS activity; points to the right of time 0 represent NOS activity after L-NAME infusion.

Results

Physiologic Data

Arterial blood gas and hemodynamic data are shown in table 2. There were no physiologically significant

Table 2. Arterial Blood Gas Data, MABP, ICP, and CMR_{O_2} at Time of Microsphere Injection

	0 min		90 min		180 min	
	Baseline	Isoflurane	Baseline	Isoflurane	Baseline	Isoflurane
pH						
Controls	7.39 ± 0.02	7.36 ± 0.02†	7.39 ± 0.02	7.38 ± 0.02	7.39 ± 0.02	7.38 ± 0.2
Indo/NAME	7.40 ± 0.01	7.35 ± 0.02†	7.36 ± 0.02	7.44 ± 0.07	7.32 ± 0.03	7.29 ± 0.04
NAME/Indo	7.40 ± 0.01	7.37 ± 0.01†	7.35 ± 0.04	7.35 ± 0.05	7.37 ± 0.03	7.39 ± 0.03
NAME/Arg	7.41 ± 0.01	7.38 ± 0.01†	7.39 ± 0.02	7.38 ± 0.03	7.43 ± 0.01	7.41 ± 0.01
Pa_{CO_2} (mmHg)						
Controls	40.3 ± 1.3	40.5 ± 1.4	41.0 ± 0.4	40.2 ± 1.0	42.3 ± 1.3	41.4 ± 1.2
Indo/NAME	39.3 ± 0.7	40.1 ± 1.2	42.5 ± 1.2	41.1 ± 1.2	41.5 ± 1.0	41.4 ± 1.3
NAME/Indo	39.1 ± 1.0	39.7 ± 0.8	39.3 ± 2.4	39.0 ± 1.6	41.0 ± 1.3	40.0 ± 0.8
NAME/Arg	40.5 ± 1.5	39.9 ± 1.4	40.4 ± 1.0	41.3 ± 1.2	40.5 ± 1.1	42.7 ± 0.8
Pa_{O_2} (mmHg)						
Controls	165 ± 20	164 ± 18	136 ± 9	142 ± 14	133 ± 15	124 ± 14
Indo/NAME	160 ± 13	162 ± 14	175 ± 11*	149 ± 14	178 ± 22	176 ± 16*
NAME/Indo	152 ± 11	179 ± 15	130 ± 18	173 ± 20†	171 ± 18	154 ± 9†
NAME/Arg	168 ± 15	194 ± 11†	183 ± 13*	192 ± 10*	173 ± 14	204 ± 16
MABP (mmHg)						
Controls	113 ± 4	114 ± 3	118 ± 2	116 ± 2	118 ± 3	116 ± 2
Indo/NAME	118 ± 4	111 ± 3	122 ± 3	116 ± 3	119 ± 3	119 ± 4
NAME/Indo	116 ± 4	107 ± 5	119 ± 2	112 ± 6	118 ± 7	114 ± 6
NAME/Arg	119 ± 5	111 ± 3†	119 ± 5	113 ± 4	119 ± 4	105 ± 6†
ICP (mmHg)						
Controls	6 ± 1	11 ± 2†	8 ± 2	13 ± 3†	10 ± 3	16 ± 4†
Indo/NAME	9 ± 2	17 ± 2†	12 ± 2	14 ± 3	7 ± 1	10 ± 1†
NAME/Indo	5 ± 2	11 ± 3†	6 ± 2	7 ± 3	4 ± 2	7 ± 2†
NAME/Arg	3 ± 1	9 ± 1†	3 ± 1	7 ± 1†	6 ± 1	10 ± 2†

Values are mean ± SEM. n = 7 control pigs; n = 8 indomethacin/L-NAME (Indo/NAME) pigs; n = 7 L-NAME/indomethacin (NAME/Indo) pigs; n = 9 L-NAME/L-Arginine (NAME/Arg) pigs. Pa_{CO_2} = arterial partial pressure of carbon dioxide; Pa_{O_2} = arterial partial pressure of oxygen; MABP = mean arterial blood pressure; ICP = intracranial pressure.

* $P \leq 0.05$ *versus* control pigs at same condition.

† $P \leq 0.05$ *versus* baseline, same group and same time.

differences in arterial blood gases between groups or within groups before or during isoflurane. MABP was maintained between 110 and 120 mmHg by using the aortic and vena caval balloons. An increase in ICP with isoflurane, however, caused a small decrease in cerebral perfusion pressure in several groups (fig. 2). Even so, cerebral perfusion pressure was maintained greater than 90 mmHg in all groups. There were minor differences in hemoglobin concentration between and within groups, but hemoglobin was maintained at or greater than 9 g/dl in all groups. Serum glucose concentration and brain temperature were maintained within the normal physiologic range in all groups. Direct examination of the brains *post mortem* revealed no significant injury associated with insertion of the intraventricular catheter or temperature probe.

Nitric Oxide Synthase Activity

Because the dose response to L-NAME in this age pig had not previously been defined, it was necessary to determine the effect of L-NAME 40 mg/kg iv on NO synthase activity in whole brain. In the four animals used to determine the effect of this dose of L-NAME on NO synthase activity, NO synthase activity decreased by 73 ± 5% within 15 min of L-NAME administration. This degree of inhibition was sustained over the duration of the experiment (fig. 1).

Cerebral Blood Flow

In control animals, isoflurane caused a consistent cerebral hyperemia in all brain regions tested, and this hyperemia did not diminish with repeated applications over time (table 3). Isoflurane-induced hyperemia was greater in hindbrain than in forebrain. Before drug ad-

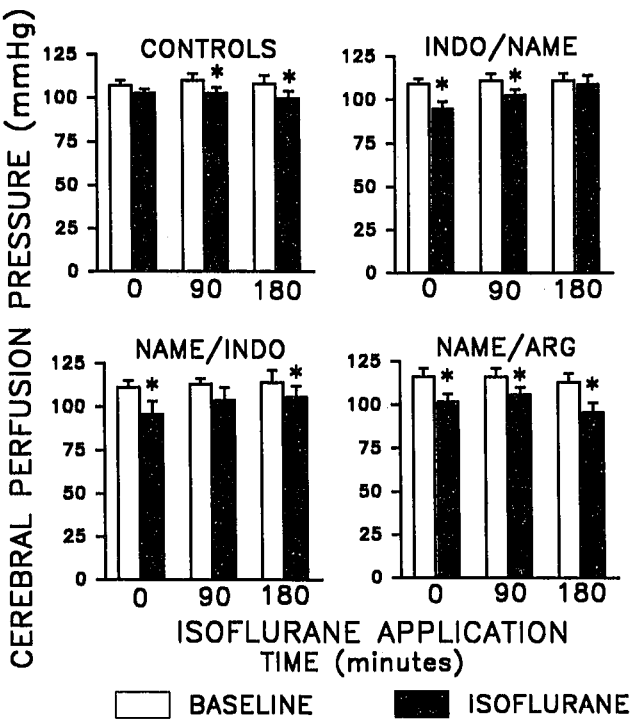

Fig. 2. Cerebral perfusion pressure (CPP) (millimeters mercury) for each experimental group at times 0, 90, and 180 min. For each group, baseline CPP was measured on pentobarbital alone (open bars), and then animals were subjected to 30-min 1 MAC isoflurane challenges, during which CPP was measured (filled bars). Isoflurane was then discontinued for 60 min before the next baseline measurements. INDO = indomethacin; NAME = N^{ω}-nitro-L-arginine methyl ester; ARG = L-arginine. Vertical lines above the bars represent the standard error for CPP. *$P < 0.05$ *versus* baseline value, same time and group.

ministration (*i.e.*, at time 0), isoflurane did not cause a statistically significant change in CMR_{O_2} (2.79 ± 0.24 to 2.34 ± 0.17 ml · min^{-1} · 100 g^{-1}; $P = 0.08$, n = 31). There were no differences in CMR_{O_2} between control animals and those animals receiving experimental treatment (table 3).

Although indomethacin had no effect on baseline CBF, L-NAME caused a 23 ± 7% decrease in baseline CBF (whole brain baseline CBF at time 90 *versus* time 0 in all animals receiving L-NAME, n = 16). Because of this finding, the data in figures 3 and 4 are presented as the percent increase in CBF with each isoflurane application. In forebrain, both indomethacin and L-NAME administration attenuated the CBF response to isoflurane (fig. 3). The hyperemic response to isoflurane returned to control levels with the administration of L-arginine in L-NAME/L-arginine animals, demonstrating reversibility of this effect. The administration of indomethacin with L-NAME did not further alter the response to isoflurane as compared with administration of either agent alone. Inhibition of isoflurane-induced hyperemia by indomethacin and L-NAME was similar in hindbrain, and L-arginine administration returned isoflurane-induced hyperemia to baseline in animals previously treated with L-NAME (fig. 4).

Discussion

We found that repetitive applications of isoflurane produced a predictable increase in CBF and that isoflurane-induced hyperemia is greater in hindbrain than in forebrain structures. With the doses of L-NAME and indomethacin used in this study, inhibition of NO synthase or cyclooxygenase resulted in marked attenuation of isoflurane-induced hyperemia. Neither mechanism involves alteration of CMR_{O_2}. In pigs treated with L-NAME, subsequent administration of L-arginine restored isoflurane-induced hyperemia. We conclude that NO and prostanoids contribute to isoflurane-induced cerebral hyperemia.

Previous studies *in vitro* suggest that volatile anesthetics may inhibit endothelium-mediated vasodilation[10] and at low concentrations may produce endothelium-dependent vasoconstriction.[3] Furthermore, isolated rat thoracic aortas continue to dilate in response to isoflurane in the absence of endothelium and in the presence of L-NAME. These data suggest that isoflurane-induced hyperemia is a direct effect and is not mediated by NO.[11] Eskinder *et al.*[24] reported that although halothane causes a large increase in cyclic guanosine 3,5-monophosphate in isolated canine cerebral vessels, this results from the selective activation of particulate guanylate cyclase without stimulation of soluble guanylate cyclase. The mechanism by which halothane stimulates particulate but not soluble guanylate cyclase in this preparation *in vitro*[24] is unclear. Our data demonstrate that isoflurane-induced hyperemia is attenuated with NO synthase inhibition. This suggests that a primary mechanism for isoflurane-induced hyperemia *in vivo* involves NO-mediated activation of soluble rather than particulate guanylate cyclase.[25] We cannot exclude, however, the possibility that stimulation of particulate guanylate cyclase in brain[26] may contribute to the residual isoflurane-induced hyperemia that is not blocked by L-NAME.

The most obvious explanation for why our results contradict data from studies *in vitro* is the use of a system *in vivo*. In addition to the endothelial cell, NO

Table 3. Cerebral Blood Flow and Cerebral Oxygen Consumption

	0 min		90 min		180 min	
	Baseline	Isoflurane	Baseline	Isoflurane	Baseline	Isoflurane
CBF						
Whole brain						
Controls	51 ± 4	107 ± 12†	57 ± 5	122 ± 10†	74 ± 10	150 ± 25†
Indo/NAME	40 ± 3*	88 ± 8†	41 ± 10	45 ± 8*	32 ± 4*	36 ± 4*
NAME/Indo	43 ± 4	80 ± 6†	33 ± 3*	45 ± 3*†	29 ± 2*	35 ± 3*†
NAME/Arg	46 ± 3	84 ± 8†	32 ± 1*	52 ± 5*†	44 ± 5*	93 ± 12†
Forebrain						
Controls	49 ± 3	97 ± 12†	54 ± 5	106 ± 10†	70 ± 10	127 ± 21†
Indo/NAME	39 ± 3*	77 ± 7†	40 ± 10	43 ± 8*	31 ± 3*	34 ± 4*
NAME/Indo	40 ± 4	68 ± 6†	31 ± 3*	40 ± 3*†	29 ± 2*	33 ± 3*
NAME/Arg	45 ± 2	71 ± 7†	31 ± 1*	44 ± 5*†	41 ± 4*	78 ± 10†
Hindbrain						
Controls	59 ± 5	157 ± 15†	70 ± 5	200 ± 16†	94 ± 15	256 ± 48†
Indo/NAME	47 ± 3	135 ± 17†	43 ± 12	53 ± 10*	38 ± 5*	47 ± 6*†
NAME/Indo	51 ± 6	130 ± 14†	41 ± 5*	67 ± 5*†	31 ± 3*	42 ± 4*†
NAME/Arg	54 ± 5	145 ± 15†	40 ± 2*	88 ± 9*†	57 ± 7	163 ± 21†
Cerebellum						
Controls	66 ± 6	168 ± 22†	80 ± 7	214 ± 22†	110 ± 21	281 ± 56†
Indo/NAME	55 ± 4	157 ± 18†	49 ± 14	66 ± 13*	46 ± 6*	59 ± 7*†
NAME/Indo	57 ± 6	143 ± 17†	48 ± 6*	75 ± 6*†	37 ± 4*	53 ± 5*†
NAME/Arg	59 ± 5	165 ± 18†	46 ± 3*	98 ± 10*†	65 ± 9	183 ± 27†
Hippocampus						
Controls	34 ± 3	73 ± 5†	39 ± 3	81 ± 6†	49 ± 9	103 ± 18†
Indo/NAME	29 ± 2	62 ± 6†	32 ± 9	33 ± 7*	22 ± 3*	24 ± 3*
NAME/Indo	29 ± 3	57 ± 5†	23 ± 3*	33 ± 3*†	20 ± 2*	24 ± 2*†
NAME/Arg	29 ± 2	55 ± 5*†	22 ± 1*	35 ± 4*†	30 ± 3	66 ± 9†
CMR_{O_2}						
Controls	3.21 ± 0.38	2.63 ± 0.39†	2.87 ± 0.36	2.48 ± 0.33	3.00 ± 0.46	2.73 ± 0.67
Indo/NAME	2.37 ± 0.30	2.02 ± 0.56	3.30 ± 0.79	1.76 ± 0.22	3.22 ± 0.18	2.87 ± 0.19
NAME/Indo	2.43 ± 0.24	2.49 ± 0.70	3.12 ± 0.11	2.72 ± 0.30	2.98 ± 0.20	2.68 ± 0.19
NAME/Arg	3.14 ± 0.37	2.28 ± 0.34†	3.15 ± 0.13	2.18 ± 0.17†	3.13 ± 0.19	2.00 ± 0.30†

Values are mean ± SEM (ml · min^{-1} · 100 g^{-1}). n = 7 control pigs; n = 8 indomethacin/L-NAME (Indo/NAME) pigs; n = 7 L-NAME/indomethacin (NAME/Indo) pigs; n = 9 L-NAME/L-Arginine (NAME/Arg) pigs. CBF = cerebral blood flow; CMR_{O_2} = cerebral oxygen consumption.

* $P \leq 0.05$ *versus* control pigs at same condition.

† $P \leq 0.05$ *versus* baseline, same group and time.

synthase has been identified in astrocytes[14] and in neurons.[15,27] Furthermore, using canine cerebral artery strips, Toda and Okamura[28] demonstrated that transmural electrical stimulation–induced relaxation is inhibited by N^G-monomethyl-L-arginine in a dose-dependent manner and that this inhibition is reversed by L-arginine. This suggests that NO may be involved in signal transduction between the vasodilator nerve and arterial smooth muscle. None of these potential sites for NO effects is present in a preparation wholly *in vitro*. In contrast to observations in isolated vessels, our data are consistent with work by Koenig *et al.* using a cranial window technique in rats and demonstrating that halothane-induced pial vessel dilation is prevented by NO synthase inhibition.[4] McPherson *et al.* demonstrated similar results *in vivo* in dogs.[29]

L-NAME and other L-arginine analogues are competitive inhibitors of NO synthase both *in vitro*[30] and *in vivo*.[31] This inhibition of NO synthase is competitively reversed with administration of L-arginine, which serves as substrate for the enzyme.[18] We found in pigs that L-NAME at a dose of 40 mg/kg iv caused a 70% inhibition of NO synthase. Despite this incomplete blockade of NO synthase, L-NAME at this dose caused significant attenuation of isoflurane-induced hyperemia, which was returned to baseline with the administration of L-arginine. Therefore, we conclude that NO is an important mediator of isoflurane-induced hyperemia. Ad-

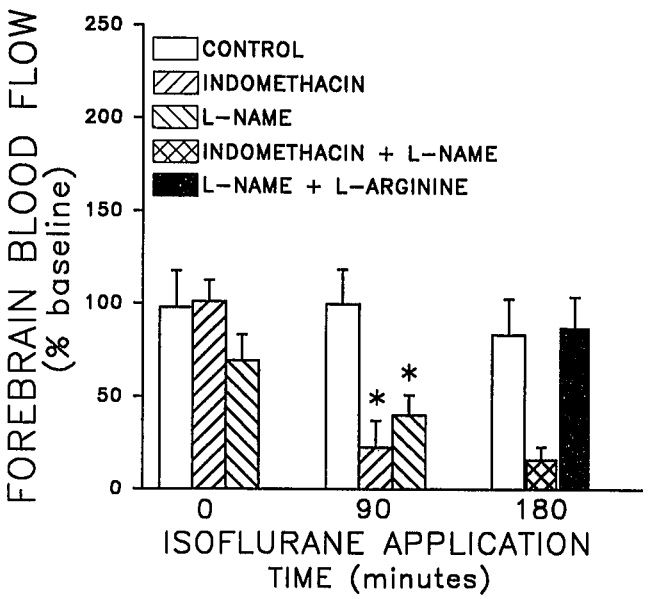

Fig. 3. Percent increase in cerebral blood flow (CBF) in response to isoflurane at times 0, 90, and 180 min in forebrain. Open bars = control group (n = 7); hatched bars rising to the right = animals receiving indomethacin (n = 8) before 90 min; hatched bars rising to the left = animals receiving N^{ω}-nitro-L-arginine methyl ester before 90 min (n = 16); cross-hatched bars = all crossover animals receiving both N^{ω}-nitro-L-arginine methyl ester and indomethacin at 180 min (n = 15); filled bars = animals receiving L-arginine after N^{ω}-nitro-L-arginine methyl ester at 180 min (n = 9). Vertical lines above the bars represent the standard error for percent increase in CBF. *$P < 0.05$ baseline CBF *versus* CBF during 1 MAC isoflurane at time 90 min as compared to baseline CBF *versus* CBF during 1 MAC isoflurane at time 0 min, by analysis of variance for each group.

ditional studies are needed to determine if using a dose of L-NAME sufficient to cause 100% inhibition of NO synthase can further attenuate or even ablate the hyperemic response to isoflurane.

We also found that indomethacin attenuated isoflurane-induced hyperemia to a similar extent as found with L-NAME. This study was not designed, however, to determine the relationship or relative significance of these two mechanisms. Our CBF data are supported by previous work demonstrating the importance of prostanoids in mediating isoflurane-induced hyperemia. For example, isoflurane administration *in vitro* may induce release of a vasodilating prostanoid in an endothelium-dependent manner.[3] NO has also been found to cause release of prostacyclin from endothelium.[32] Although we are unable to conclude whether there is an interaction between the prostanoid and NO systems in mediating this hyperemic response, there is evidence that the two systems are closely interactive. For example, Ignarro and Kadowitz suggested that arachidonic acid may increase concentrations of cyclic guanosine 3,5-monophosphate[33] and that this effect is inhibited by methylene blue but not by indomethacin.[33] Shimokawa *et al.*[34] likewise demonstrated that exogenously administered prostacyclin causes relaxation of porcine coronary arteries, an effect that is potentiated in the presence of endothelium. This potentiation was inhibited by oxyhemoglobin but not by indomethacin, suggesting that prostacyclin facilitates the release of NO. Although there is no direct evidence that prostanoids cause the release of NO, in view of previous studies it seems possible that the two are linked in mediating vasodilation.

Because we were interested in evaluating the CBF response to isoflurane, it was necessary to use a basal anesthetic to maintain adequate levels of anesthesia between

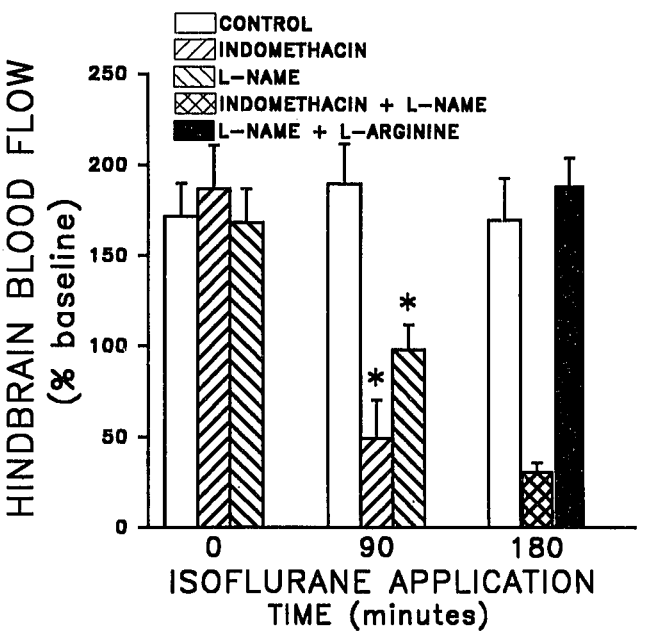

Fig. 4. Percent increase in cerebral blood flow (CBF) in response to isoflurane at times 0, 90, and 180 min in hindbrain. Open bars = control group (n = 7); hatched bars rising to the right = animals receiving indomethacin (n = 8) before 90 min; hatched bars rising to left = animals receiving N^{ω}-nitro-L-arginine methyl ester before 90 min (n = 16); cross-hatched bars = all crossover animals receiving both N^{ω}-nitro-L-arginine methyl ester and indomethacin at 180 min (n = 15); filled bars = animals receiving L-arginine after N^{ω}-nitro-L-arginine methyl ester at 180 min (n = 9). Vertical lines above bars represent the standard error for percent increase in CBF. *$P < 0.05$ baseline CBF *versus* CBF during 1 MAC isoflurane at time 90 min as compared to baseline CBF *versus* CBF during 1 MAC isoflurane at time 0 min, by analysis of variance for each group.

isoflurane applications. Our data do not demonstrate a consistent decrease in CMR_{O_2} with isoflurane. This finding is consistent with data in rabbits that demonstrated that the decrease in CMR_{O_2} resulting from isoflurane is related to baseline CMR_{O_2}.[2] Therefore, because pentobarbital is known to decrease CMR_{O_2},[35] it is not surprising that there was no further statistically significant decrease in CMR_{O_2} with isoflurane administration. The work by Drummond *et al.*[2] demonstrated that cerebral hyperemia produced by a volatile anesthetic is directly related to the degree of CMR_{O_2} depression produced by that volatile agent—thus a persistent "coupling" of CBF and metabolism. Therefore, although the basal pentobarbital anesthetic may have limited the hyperemic response to isoflurane, CMR_{O_2} was similarly depressed in all groups as a result of the pentobarbital and was not affected by experimental treatment. Therefore the pentobarbital infusion in our study should not have affected CBF data disproportionately between groups and so does not alter our conclusions.

We found that the increase in CBF in response to isoflurane was greater in hindbrain than forebrain (figs. 3 and 4). These regional differences in isoflurane reactivity are consistent with other stimuli known to produce cerebral hyperemia, such as hypercapnia,[20] and correlate with regional differences in NO synthase immunoreactivity. NO synthase in rats appears to be concentrated in the cerebellar molecular and granule cell layers, hippocampus, and superior and inferior colliculi.[15] The combination of L-NAME and indomethacin did not further alter isoflurane-induced hyperemia as compared with use of either agent alone. We cannot rule out the possibility that there are additional mechanisms for isoflurane-induced hyperemia that have not been recognized and that may be regional.

Our study was not designed to determine precisely how isoflurane stimulated the production or release of NO. It seems likely, however, that this response did not arise solely from effects on the endothelial cell, because in isolated vessels isoflurane-induced vasorelaxation occurs in the absence of endothelium.[3] In fact, volatile anesthetics may inhibit endothelium-mediated vasorelaxation.[10] Recently, Nozaki *et al.* studied the origin and distribution of perivascular nerve fibers expressing NO synthase immunoreactivity.[27] In addition to showing species differences in the distribution of NO synthase–containing fibers between rats and humans, they demonstrated a 75% reduction in these fibers by sectioning postganglionic fibers arising from the sphenopalatine ganglion in rats. It seems likely that these NO synthase–containing fibers are widely distributed in pigs because of the diffuse effects of NO synthase inhibition on basal CBF.[36] It is possible that isoflurane stimulates release of NO from these fibers, which then diffuses to effector sites on vascular smooth muscle; similar sectioning of these postganglionic fibers would be required to prove such a hypothesis. Other potential mechanisms of isoflurane-induced NO effects include inhibitory effects on cyclic guanosine 3,5-monophosphate phosphodiesterase or stimulatory effects on perivascular astrocytes, also known to contain NO synthase.[14] In addition, isoflurane may produce a direct vasodilatory effect with a CBF-dependent increase in NO production, a mechanism that we cannot exclude given the current results. It seems unlikely that L-NAME attenuated isoflurane-induced hyperemia by a direct vasoconstrictive effect, limiting the ability of cerebral vessels to dilate, because cerebral vessels continue to dilate in response to other stimuli such as hypoxia[37] and hypoglycemia[38] after NO synthase inhibition. Similarly, after indomethacin cerebral vessels continue to dilate in response to hypoxia.[39,40]

In summary, isoflurane caused cerebral hyperemia in pigs, an effect that could be attenuated by NO synthase inhibition, and returned toward baseline with administration of L-arginine. As previously demonstrated,[3] isoflurane-induced cerebral hyperemia also was mediated by release of a vasodilating prostanoid. These two systems may be closely interactive, but further investigation is required to determine if they are two consecutive steps in isoflurane-induced hyperemia or if the mechanisms are entirely separate.

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