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Isoflurane-induced Cerebral Hyperemia Is Partially Mediated by Nitric Oxide and Epoxyeicosatrienoic Acids in Mice In Vivo

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Background: **Despite intense investigation, the mechanism of isoflurane-induced cerebral hyperemia is unclear. The current study was designed to determine the contributions of neuronal nitric oxide synthase, prostaglandins, and epoxyeicosatrienoic acids to isoflurane-induced cerebral hyperemia.**

Methods: **Regional cerebral cortical blood flow was measured with laser Doppler flowmetry during stepwise increases of isoflurane from 0.0 to 1.2, 1.8, and 2.4 vol% end-tidal concentration in α-chloralose–urethane-anesthetized, C57BL/6 mice before and 45 min after administration of the neuronal nitric oxide synthase inhibitor 7-nitroindazole (7-NI, 40 mg/kg, intraperitoneal), the cyclooxygenase inhibitor indomethacin (INDO, 10 mg/kg, intravenous), and the cytochrome P450 epoxygenase inhibitor N-methylsulfonyl-6-(2-proparglyoxyphenyl)hexanoic acid (PPOH, 20 mg/kg, intravenous).**

Results: **Isoflurane increased regional cerebral cortical blood flow by 9 ± 3, 46 ± 21, and 101 ± 26% (SD) at 1.2, 1.8, and 2.4 vol%, respectively. The increases in regional cerebral cortical blood flow were significantly (*$P < 0.05$) smaller after 7-NI (5 ± 6, 29 ± 19*, 68 ± 15%*) or PPOH (4 ± 8, 27 ± 17*, 67 ± 30%*), but not after administration of INDO (4 ± 4, 33 ± 18 [NS], 107 ± 35% [NS]). The effect of combined treatment with 7-NI, PPOH, and INDO was not additive and was equal to that of either 7-NI or PPOH alone (5 ± 5, 30 ± 12*, 76 ± 24%*). Chronic treatment of mice for 5 days with 7-NI (2 × 40 mg/kg, intraperitoneal) produced similar decreases in regional cerebral cortical blood flow as those seen with acute administration. Neither PPOH nor INDO conferred a significant additional block of the hyperemia in these animals.**

Conclusions: **Nitric oxide and epoxyeicosatrienoic acids contribute to isoflurane-induced hyperemia. However, only approximately one third of the cerebral hyperemic response to isoflurane is mediated by autacoids. The remaining part of this response appears to be mediated by a direct action of isoflurane on smooth muscle by some yet-unknown mechanism.**

ISOFLURANE has been shown to cause cerebral hyperemia in dogs, pigs, rabbits, rats, and mice.[1–5] Cerebral vascular diameter is controlled by neurohumoral, myogenic, metabolic, and endothelium-dependent factors. Endothelial cells synthesize and release vasodilators such as nitric oxide (NO), prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF).[6,7] Nitric oxide has been reported to participate in isoflurane-induced cerebral vasodilatation *in vivo*[4,8,9] and is produced under normal physiologic conditions by the two constitutive isoforms of nitric oxide synthase (NOS), endothelial NOS (eNOS), and neuronal NOS (nNOS). In a previous study in mice,[5] we tried to delineate the contribution of eNOS and nNOS to isoflurane-induced cerebral hyperemia by studying its effects in nNOS gene-deficient (knockout) mice. We found that the hyperemia was present to the same extent in nNOS knockout and wild-type mice. These results indicate that the hyperemic response to isoflurane is dependent on eNOS. The unspecific NOS inhibitor L-NNA attenuated the response to isoflurane more in the knockout mice than in wild-type mice at lower isoflurane concentrations but had no effect on isoflurane-induced cerebral hyperemia at higher isoflurane concentrations. In addition to eNOS, other mediators might play a role in cerebral vasodilation. It has been reported that in pigs, isoflurane-induced cerebral hyperemia is also dependent on NO, but also on prostaglandins.[4] Recently, epoxyeiscosatrienoic acids (EETs), cytochrome P450-derived metabolites of arachidonic acid, have been identified as EDHF and implicated in reactive hyperemia.[10,11] This study examined the relative contributions of NO, prostaglandins, and EETs to the cerebral hyperemic effect of isoflurane by measuring cerebrocortical blood flow (rCBF) with laser Doppler flowmetry in mice during stepwise increases in the inhalational concentration of isoflurane.

Methods

The experimental procedures and protocols used in this investigation were reviewed and approved by the Institutional Animal Care and Use Committee (Medical College of Wisconsin, Milwaukee, Wisconsin). All procedures conformed to the *Guiding Principles in the Care and Use of Animals*[12] of the American Physiologic Society and were in accordance with the *Guide for the Care and Use of Laboratory Animals*.[13]

Surgical Procedures and Cerebral Blood Flow Monitoring

Experiments were performed using C57BL/6 mice (Taconic Farms, Germantown, NY) as a continuation of

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former investigations in the same species.[5] Mice were anesthetized by α-chloralose–urethane (50/500 mg/kg; intraperitoneal), paralyzed (pancuronium, 0.4 mg/kg, intraperitoneal), tracheotomized, and artificially ventilated with a small animal ventilator (SAR-830; CWE, Ardmore, PA) with 30% O_2 in N_2. Anesthesia was maintained by repetitive administration of small boluses of α-chloralose–urethane (5/50 mg/kg, intraperitoneal) as necessary. End-tidal carbon dioxide tension (ET_{CO_2}) was measured by microcapnometry (Capstar-100; IITC Inc., Woodland Hills, CA) that reliably detected carbon dioxide at sampling flow rates of 15–20 ml/min. This sampling rate has been reported not to interfere with the respiration of the animals.[14] Body temperature was maintained at 37 ± 1°C using a thermostat-controlled water-circulated heating mat. The right femoral artery was cannulated with PE-10 tubing to facilitate the measurement of arterial pressure and arterial blood gases. Arterial partial pressure of oxygen (P_{O_2}), partial pressure of carbon dioxide (P_{CO_2}), and pH were measured with a blood gas analyzer (ABL-300; Radiometer, Denmark). The femoral vein was cannulated for the infusion of drugs. Inspired oxygen and isoflurane concentrations were continuously monitored (POET II; Criticare Systems, Inc., Milwaukee, WI). Regional cerebrocortical blood flow (rCBF) was determined by laser Doppler flowmetry (PF3; Perimed, Sweden). The techniques used for monitoring cerebrocortical blood flow in mice with laser Doppler flowmetry have been described previously.[3] The head of the mouse was placed in a stereotaxic apparatus (Model 900; David Kopf, Tujunga, CA). After skin incision and exposing the skull, the laser Doppler flow probe was positioned using a micromanipulator so that there were no visible pial vessels in the field of measurement. The probe did not touch the cranium, and a drop of mineral oil was used to improve optical coupling between the probe and the tissue. ET_{CO_2}, mean arterial blood pressure (MAP), rCBF, and isoflurane concentrations were recorded on an eight-channel polygraph (Astro-Med, Inc., West Warwick, RI) or computer data acquisition system (Mac lab/8; AD Instruments, Medford, MA). After completion of surgery, a 30-min equilibration period was allowed, and baseline measurements were recorded. Regional cortical cerebral blood flow was recorded with two laser Doppler flow probes on both hemispheres.

Experimental Protocol

The mice were randomly assigned to seven groups, and experiments were performed according to the general protocol. The groups were as follows: group 1, vehicle (VEH, saline) treatment (n = 5, number of animals); group 2, 7-nitroindazole (7-NI, 40 mg/kg, intraperitoneal, n = 6); group 3, N-methylsulfonyl-6-(2-proparglyoxyphenyl) hexanoic acid (PPOH, 20 mg/kg, intravenous, n = 6); group 4, indomethacin (INDO, 10 mg/kg, intravenous, n = 5); group 5, 5-day chronic treatment with 7-NI (2×40 mg · kg^{-1} · day^{-1}, intraperitoneal, n = 6); group 6, chronic 7-NI treatment (2×40 mg · kg^{-1} · day^{-1}, intraperitoneal) combined with acute PPOH (20 mg/kg, intravenous, n = 6); group 7, chronic 7-NI (2×40 mg · kg^{-1} · day^{-1}, intraperitoneal) combined with acute INDO (10 mg/kg, intravenous) (n = 5).

After obtaining baseline measurements, the inhaled concentration of isoflurane was increased in a stepwise manner from 0.0 (baseline) to 1.2, 1.8, and 2.4 vol% end-tidal concentration. Each concentration of isoflurane was maintained for 15 min, and the change in rCBF was recorded. Then, we infused either the selective neuronal NOS inhibitor 7-NI (40 mg/kg, intraperitoneal), the P450 enzyme epoxygenase inhibitor PPOH (20 mg/kg, intravenous), or the cyclooxygenase inhibitor INDO (10 mg/kg, intravenous). After 45 min, the concentration of isoflurane was increased again from 0.0 (baseline) to 1.2, 1.8, and 2.4 vol% and maintained for 15 min at each level. To maintain the arterial blood pressure throughout the experimental period, methoxamine, an α_1-adrenergic receptor agonist, was infused intravenously. The rate of infusion was varied in the range of 0.5–2 μg/min, to maintain MAP at the same level. It has been reported that adrenergic agonists do not affect rCBF.[15]

Assessment of Nitric Oxide Synthase Activity

In separate experiments, mice were euthanized, the brains were removed, and the cerebral cortex was separated and rapidly frozen in liquid nitrogen and stored at −70°C until NOS activity was determined. NOS activity was measured by the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline according to the high-performance liquid chromatography method originally described by Carlberg.[16] The cerebral cortical tissue was homogenized in 20 mM HEPES buffer (pH 7.4). The homogenate was then centrifuged at 9,000*g* for 10 min at 4°C. The supernatant (each sample including 150 μg protein determined by protein assay according to the Bradford Method, Biorad, CA) was incubated with [^{3}H]L-arginine (20 mM HEPES buffer, 0.2 μCi, 100 μl, containing 2 mM $CaCl_2$, 1 mM NADPH, 1.25 μg/ml calmodulin, 2.5 μM FAD, 1 μM FMN, and tetrahydrobiopterin for 5 min at 37°C in the presence or absence of 10 and 100 μM 7-NI or L-NA and 100 μM L-NAME. The reaction was stopped by adding 20 mM EDTA solution, 50 μl (pH 5.5), frozen in liquid nitrogen. Products were separated by high-performance liquid chromatography on an LC-18 DB column (Supelco, Bellefonte, PA) at a flow rate of 1.5 ml/min. Products were monitored using an on-line radioactive flow detector (A-100; Radiomatic Instruments, Median, CT). Results were expressed as produced citrulline (pmol · mg $protein^{-1}$ · min^{-1}).

Table 1. Mean Arterial Blood Pressure (MAP) and Blood Gas Analysis in Experimental Groups

	7-NI		PPOH		INDO		Vehicle	
	Before	After	Before	After	Before	After	Before	After
MAP (mmHg)	70 ± 7	71 ± 7	73 ± 6	74 ± 7	69 ± 6	80 ± 9*	70 ± 7	65 ± 4
pco_2 (mmHg)	35 ± 5	35 ± 3	35 ± 5	34 ± 3	38 ± 4	32 ± 4*	35 ± 5	31 ± 5
po_2 (mmHg)$_0$	144 ± 24	151 ± 16	149 ± 21	152 ± 20	129 ± 21	158 ± 18	148 ± 10	161 ± 14
pH	7.38 ± 0.40	7.38 ± 0.04	7.38 ± 0.04	7.38 ± 0.04	7.40 ± 0.10	7.35 ± 0.06	7.31 ± 0.09	7.32 ± 0.05

Data are mean ± SD.

*Significantly ($P < 0.05$) different from control value "before."

7-NI = 7-nitroindazol (n = 6); PPOH = *N*-methylsulfonyl-6-(2-proparglyoxyphenyl)hexanoic acid (n = 6); INDO = indomethacin (n = 5); vehicle = saline (n = 5).

Drugs

Isoflurane was purchased from Abbott Laboratories (North Chicago, IL). Urethane, α-chloralose, pancuronium bromide, $CaCl_2$, calmodulin, HEPES, NADPH, FAD, FMN, EDTA, tetrahydrobiopterin, 7-NI (7-nitroindazol), L-NA (*N*-nitro-L-arginine), and L-NAME (*N*-nitro-L-arginine methyl ester) were purchased from Sigma Chemical Co. (St. Louis, MO), and PPOH (N-methylsulfonyl-6-(2-proparglyoxyphenyl)hexanoic acid) was synthesized and kindly provided by John R. Falck, Ph.D., D.I.C. (Professor, Robert A. Welch Distinguished Chair in Chemistry, Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas). [^{3}H]L-arginine was purchased from Amersham Laboratories (Piscataway, NJ).

Data Analysis and Statistics

The values obtained by measuring laser Doppler flow in both hemispheres in each animal were averaged to yield one value. Baseline rCBF was determined from 3-min periods under steady state conditions before and 45 min after drug treatment. The rCBF response to in-

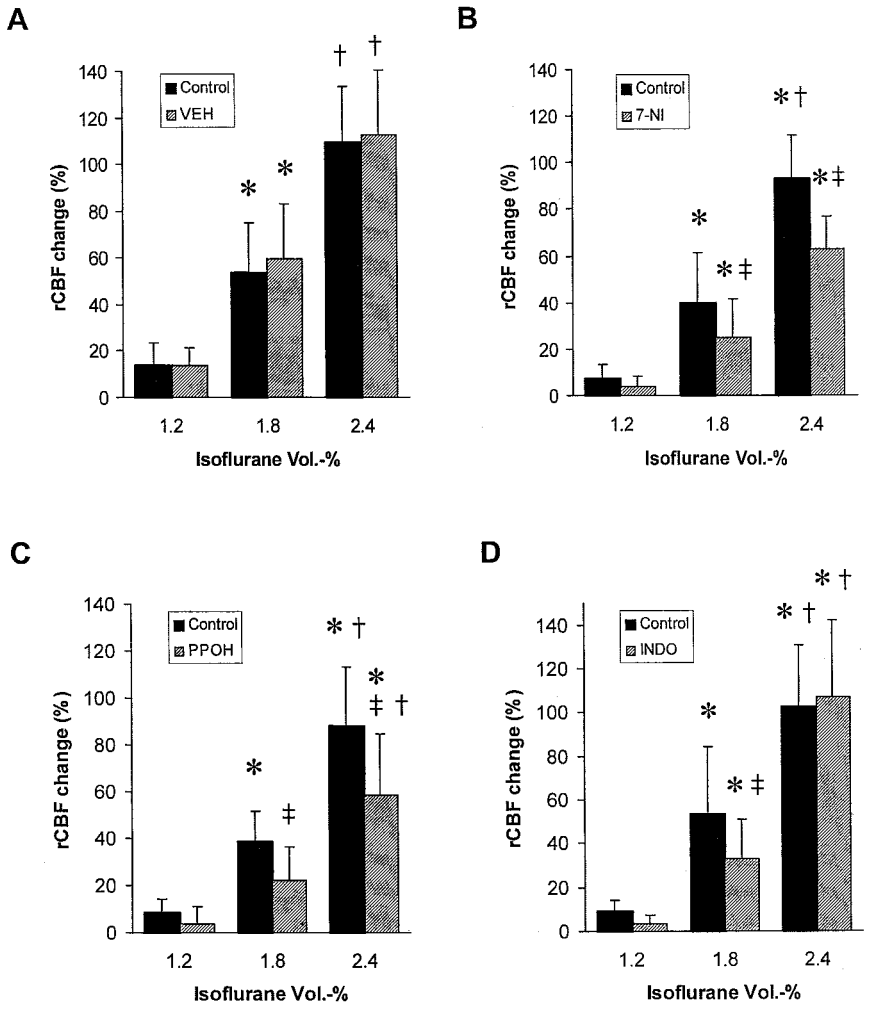

Fig. 1. Effect of isoflurane on regional cerebral blood flow (rCBF) in C57BL/6 wild-type mice before (closed column) and 45 min after (hatched column) the administration of drugs: (*A*) effect of vehicle (0.9% saline); (*B*) 7-nitroindazole (7-NI, 40 mg/kg, intraperitoneal), a neuronal NOS inhibitor; (*C*) indomethacin (INDO, 10 mg/kg, intravenous), a nonselective cyclooxygenase inhibitor; (*D*) N-methylsulfonyl-6-(2-proparglyoxyphenyl)hexanoic acid (PPOH, 20 mg/kg, intravenous), a cytochrome P450 expoxygenation inhibitor. The rCBF response was measured by laser Doppler flowmetry in the cerebral cortex *in vivo* during stepwise increases of isoflurane from 0.0 to 1.2, 1.8, and 2.4 vol% end-tidal concentration in α-chloralose–urethane-anesthetized, paralyzed, artificially ventilated mice. Forty-five minutes were interspersed before the stepwise increase of isoflurane concentration was repeated in the presence of the respective drug. Vehicle and indomethacin (at 2.4 vol%) had no significant impact on the isoflurane-induced cerebral hyperemia, whereas 7-NI and PPOH attenuated the response by 29%. Data are expressed as mean ± SD; n = 6–7 animals. *Significantly ($P < 0.05$) different from 0.0 or 1.2 vol% isoflurane. †Significantly ($P < 0.05$) different from 1.8 vol% isoflurane. ‡Significantly ($P < 0.05$) different from control.

creasing isoflurane concentration was obtained from the last 3 min of the rCBF record of the 15-min equilibration period allowed at each anesthetic concentration. The rCBF response was expressed as percent change from baseline value. Data were expressed as mean ± SD. Statistical significance of the effect of isoflurane at the three concentrations of isoflurane before and after treatment with L-NAME, PPOH, or 7-NI was tested with two-way analysis of variance with repeated measures. $P < 0.05$ was considered to be significant.

Results

Baseline values of mean arterial pressure, arterial blood pH, P_{CO_2}, and P_{O_2} before and after drug treatment in experimental groups of mice are presented in table 1. There was a small, significant increase in baseline MAP and decrease in baseline P_{CO_2} values after treatment with indomethacin. There were no significant differences in any other variables among experimental groups.

The effects of isoflurane before and after the administration of vehicle, 7-NI, PPOH, or INDO are presented in figure 1. In the following, all rCBF baseline are presented as percent increase at 1.2, 1.8, and 2.4% isoflurane concentration, respectively, unless noted otherwise. During the control period, rCBF dose-dependently increased rCBF by 13 ± 10, 54 ± 21, 109 ± 24% in vehicle-treated mice (fig. 1A). rCBF rose to identical values during the second exposure to isoflurane (fig. 1A). In mice treated with 7-NI (group 2), rCBF increased during the first exposure to isoflurane by 7 ± 6, 40 ± 21, 93 ± 19% (fig. 1B). Subsequent administration of 7-NI significantly attenuated the rCBF response to isoflurane by 4 ± 4, 25 ± 16, 63 ± 14% (fig. 1B). In mice assigned to group 3, rCBF increased dose-dependently by 8 ± 6, 39 ± 13, 88 ± 25% (fig. 1C) before and by 4 ± 7, 22 ± 14, 59 ± 26% after administration of PPOH (fig. 1C). In group 4 mice, isoflurane increased rCBF by 9 ± 5, 53 ± 31, 102 ± 29% during the control period. After treatment with indomethacin, a significant effect on the response to isoflurane, 8 ± 6, 39 ± 13, 88 ± 25%, could be observed at 1.8% isoflurane concentration (significantly different from control) but not at 2.4%.

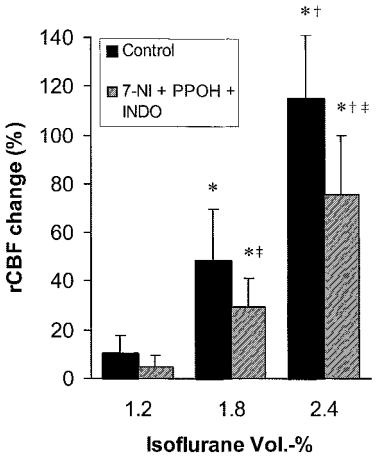

Fig. 2. Combined effect of inhibition of nNOS, cyclooxygenase, and P450 epoxygenase on isoflurane-induced cerebral hyperemia. Data are expressed as mean ± SD; n = 5 animals. *Significantly ($P < 0.05$) different from 0.0 or 1.2 vol% isoflurane. †Significantly ($P < 0.05$) different from 1.8 vol% isoflurane. ‡Significantly ($P < 0.05$) different from control.

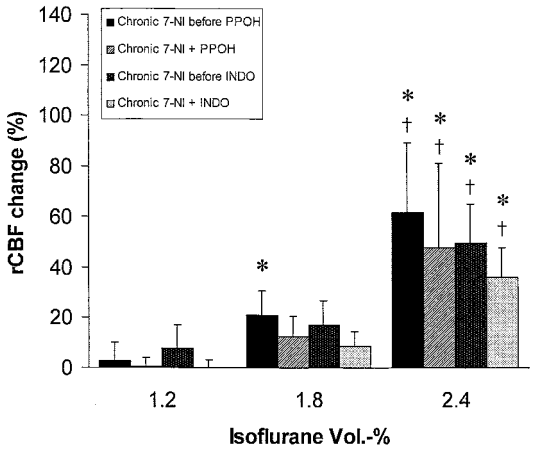

Fig. 3. Effect of chronic blockade of nNOS with 7-NI (2 × 40 mg/kg, intraperitoneal) on hyperemic response to isoflurane before and after administration of indomethacin (INDO, 10 mg/kg, intravenous) or N-methylsulfonyl-6-(2-proparglyoxyphenyl)hexanoic acid (PPOH). Data are expressed as mean ± SD; n = 5–6 animals. *Significantly ($P < 0.05$) different from 0.0 or 1.2 vol% isoflurane. †Significantly ($P < 0.05$) different from 1.8 vol% isoflurane. ‡Significantly ($P < 0.05$) different from control.

The effect of isoflurane before and after combined administration of 7-NI, PPOH, and INDO is presented in figure 2. Isoflurane increased rCBF dose dependently by 10 ± 9, 48 ± 16, 115 ± 29% (fig. 2) during the control period and by 5 ± 5, 30 ± 12, 76 ± 24% after combined administration of these inhibitors, respectively.

To further determine the efficacy of acute NOS inhibition and the relative importance of prostaglandins and EETs in mediating the isoflurane-induced cerebral hyperemia, we tested the hypothesis that chronic treatment with 7-NI might be more efficacious than acute blockade

Table 2. NOS Activity Assay of Mouse Brain and Inhibition by Various NOS Inhibitors

		Activity (pmol · min^{-1} · mg^{-1})	Percent Inhibition *vs.* Control (%)
Control		122 ± 2	0 ± 2
7-NI	10 μM	97 ± 30	21 ± 24
—	100 μM	28 ± 2*†	77 ± 2*†
L-NA	10 μM	91 ± 8	25 ± 7
—	100 μM	11 ± 5*†	91 ± 4*†
L-NAME	100 μM	1 ± 1*†	99 ± 1*†

Data are mean ± SD. *Significantly ($P < 0.05$) different from control; † Significantly ($P < 0.05$) different from 10 μM 7-NI or L-NA.

Control = no inhibitor; 7-NI = 7-nitroindazol; L-NA = *N*-nitro-L-arginine; L-NAME = *N*-nitro-L-arginine methyl ester.

(1 × 40 mg/kg, intraperitoneal) or that up-regulation of the synthesis of prostaglandin and EETs might compensate for the loss of NO availability. To this end, mice were treated for 5 days with 7-NI (2 × 40 mg/kg, intraperitoneal), and the rCBF response before and after acute blockade of the synthesis of EETs or prostaglandins was determined. Chronic treatment with 7-NI had the same effect to blunt the rCBF response to isoflurane as acute administration of 7-NI (fig. 3). Subsequent administration of PPOH or INDO had no additional effect on the hyperemic response to isoflurane.

Data for the inhibitory effect of 7-NI as compared to those of L-NA (*N*-nitro-L-arginine) and L-NAME (*N*-nitro-L-arginine methyl ester) on brain NOS catalytic activity *in vitro* are given in table 2.

Discussion

Nitric oxide has been implicated to play an important role on the cerebral vasodilator response to volatile anesthetics such as halothane and isoflurane.[4,5,8,17,18] In the brain, the two constitutive isoforms of NOS, *i.e.*, eNOS and nNOS, are distributed in various cell types, including the endothelium,[19] neurons,[20] astrocytes,[21] and perivascular nerves.[22,23] The abundant presence of nNOS in the brain implies an important role of nNOS in regulating the cerebral circulation, and in the cerebral hyperemic response to isoflurane. Indeed, in the current investigation, blockade of nNOS by the selective nNOS inhibitor 7-NI inhibited isoflurane-induced rCBF increases by approximately 30%, indicating an important role for nNOS in this response.

However, in a previous study,[5] we found that knockout of nNOS had little effect on the hyperemic effect of isoflurane. We also found that the rCBF response to isoflurane in wild-type mice seemed to be dependent on both nNOS and eNOS. This raised the possibility that up-regulation of eNOS may compensate for the loss of nNOS in the knockout mice. However, blockade of eNOS activity with L-NA had no effect on isoflurane-induced cerebrovasodilation. These results, taken together, suggested that the response to isoflurane may involve the release of other vasoactive mediators or may be due to direct effects of isoflurane. Therefore, nNOS knockout mice were not included in this study because we wanted to study the effects of pharmacological inhibition of nNOS in wild-type mice and to explore the role of NOS-independent mediators in the genetically unchanged animal.

Other mediators may include metabolites of arachidonic acid, such as prostaglandins or EETs derived from the cyclooxygenase[4] or cytochrome P450 epoxygenation pathway.[10] To examine this possibility in the current studies, we also inhibited the enzymes producing prostaglandins and EETs in separate groups of mice. Inhibition with the selective P450 epoxygenation enzyme inhibitor PPOH[24] was equally effective as blockade of NO in inhibiting the rCBF response to isoflurane at 1.8 and 2.4 vol% by approximately 30%, whereas inhibition of the formation of prostaglandins by indomethacin, a cyclooxygenase I and II inhibitor, had much less effect. Taken together, these results support a role for both NO and EETs in mediating isoflurane-induced rCBF increase, but only a minor role for prostaglandins. Combined blockade with all three enzyme inhibitors did not afford any additional blockade of the response to isoflurane over that seen with 7-NI or PPOH alone. These results suggest that NO and EETs are not functionally independent of each other and that either their pathways are arranged in parallel[25] or they act on a common second messenger pathway to cause vasodilation—likely activation of K channels—to mediate the hyperemic response to isoflurane. The data also indicate that isoflurane must have a direct effect on vascular smooth muscle cells since combined blockade of NO, EETs, and prostaglandins—at the doses used— only reduced the response to isoflurane by 30%. It is also possible that higher concentration of inhibitors would produce a dose-dependent further attenuation of isoflurane-induced cerebral hyperemia; however, it is simplistic to believe that there is a linear relation of percent inhibition of enzyme activity and resultant attenuation of vasodilator response.

An alternative possibility is that the concentrations of the inhibitors used in the current study were not sufficient to completely block the respective enzyme activities. To exclude this contention, brain NOS activity was measured *ex vivo* with radio-labeled L-arginine to L-citrulline conversion after treatment with 7-NI and the nonselective eNOS and nNOS inhibitors, L-NA and L-NAME, at concentrations of 10 and 100 μM. The conversion of arginine to citrulline was inhibited by 77 ± 2% by 7-NI at a concentration of 100 μM, in good agreement with our previous determinations of nNOS inhibition by 7-NI of 70 ± 7% *in vivo* in rats that were treated with the same dosage as used in the current investigation in mice.[26] The administration of a higher dose of indomethacin was not considered since the applied dose of indomethacin (10 mg/kg intravenous) already increased MAP (table 1).

To test whether chronic inhibition of nNOS might change the relative importance of prostaglandins or EETs by up-regulating their enzyme activities, we repeated the experiments with acute administration of PPOH and INDO in animals treated chronically with 7-NI. Under these conditions, blockade of neither prostaglandins nor EETs decreased rCBF response to isoflurane more than did 7-NI. These results suggest that prostaglandins or EETs are not up-regulated following chronic nNOS blockade with 7-NI. However, we did not investigate the effects of additional blockade of eNOS in these animals, and eNOS might have been up-regulated and able to

confer cerebrovascular hyperemia, a possibility that remains to be determined in future investigations. Upregulation of the prostaglandin and EET pathways may only be induced after blockade of all NOS pathways.

While an important role of NO in isoflurane-induced cerebral vasodilatation *in vivo* is strongly suggested by previous[4,5,8,18] and the current investigations, the mechanism of involvement of NO in this response remains unclear. It has been shown that isoflurane does not directly alter NOS enzyme activity in rat brain homogenates.[27] On the other hand, isoflurane potentiates glutamate receptor–stimulated NO synthesis in primary culture of cerebral neurons.[28]

It has been suggested that isoflurane causes a greater increase in CBF when the metabolic depressant effect of isoflurane is attenuated by prior administration of barbiturate or propofol in both animals and humans.[1,29] Therefore, in the current study, we used isoflurane superimposed on α-chloralose–urethane anesthesia, to minimize the metabolism-related decrease in CBF during the exposure to isoflurane. However, this may lead to changes in baseline rCBF and responsiveness to isoflurane. In pilot experiments, we determined that anesthesia with α-chloralose–urethane does not alter baseline rCBF and augments rCBF response to isoflurane no more than 10%.

In summary, isoflurane at 1.2, 1.8, and 2.4 vol% increased rCBF in mice in a dose-dependent manner, and this increase was significantly attenuated by either PPOH or 7-NI but not by indomethacin. The effects of combined blockade of NO, prostaglandins, and EETs formation were not additive. It is concluded that isoflurane-induced hyperemia is mediated by NO and EETs acting as interrelated pathways, without a substantial contribution of prostaglandins. Only approximately one third of the isoflurane-induced hyperemia is mediated by the autacoids, NO, and EETs, and the remaining part of the rCBF increase in mice is probably related to direct smooth muscle relaxation.

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