

Periischemic Cerebral Blood Flow (CBF) Does Not Explain Beneficial Effects of Isoflurane on Outcome from Near-complete Forebrain Ischemia in Rats

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Background: Isoflurane improves outcome from near-complete forebrain ischemia in rats compared with fentanyl-nitrous oxide (N₂O). Sympathetic ganglionic blockade with trimethaphan abolishes this beneficial effect. To evaluate whether anesthesia-related differences in cerebral blood flow (CBF) may explain these findings, this study compared regional CBF before, during, and after near-complete forebrain ischemia in rats anesthetized with either isoflurane (with and without trimethaphan) or fentanyl-nitrous oxide.

Methods: Fasted, normothermic isoflurane anesthetized Sprague-Dawley rats were prepared for near-complete forebrain ischemia (10 min of bilateral carotid occlusion and mean arterial pressure = 30 mmHg). After surgery, rats were anesthetized with either 1.4% isoflurane (with or without 2.5 mg of trimethaphan intravenously at onset of ischemia) or fentanyl-nitrous oxide (25 μ g \cdot kg⁻¹ \cdot h⁻¹ \cdot 70% N₂O⁻¹). Regional CBF was determined (¹⁴C-iodoantipyrine autoradiography) before ischemia, 8 min after onset of ischemia, and 30 min after onset of reperfusion.

Results: Regional CBF did not differ significantly among groups at any measurement interval. Ischemia caused a marked flow reduction to 5% or less of baseline ($P < 0.001$) in selectively vulnerable regions, such as the cortex, caudoputamen and hippocampus, whereas flow in the brain stem and cerebellum was preserved. Reperfusion at 30 min was associated with partial restoration of flow to 35–50% of baseline values in ischemic structures.

Conclusions: The results indicate that improved histologic-behavioral outcome provided by isoflurane anesthesia cannot be explained by differential vasodilative effects of the anesthetic states before, during, or after severe forebrain ischemia. This study also shows severe postischemic delayed hypoperfusion that was not affected by choice of anesthetic or the presence of trimethaphan. Mechanisms other than effects on periischemic CBF must be responsible for beneficial effects of isoflurane in this model. (Key words: Brain; fentanyl; ganglionic blockade; isoflurane; nitrous oxide.)

ISOFLURANE is known to alter outcome after ischemic brain injury.¹⁻⁴ In a recent study of severe forebrain

ischemia, isoflurane anesthetized rats had better histologic outcome than those administered fentanyl-nitrous oxide (N₂O). The beneficial effect of isoflurane, however, was abolished when the sympathetic ganglionic blocking agent trimethaphan was administered at onset of ischemia.⁵ It was suggested that differences in anesthesia-related periischemic cerebral blood flow (CBF) might be responsible, at least in part, for the protective effects of the volatile anesthetic isoflurane.

Studies of CBF during near-complete forebrain ischemia have consistently found flow to be reduced to less than 5% of the control value.^{6,7} However, the effects of anesthetic agents on intraischemic or postischemic CBF after near-complete forebrain ischemia are largely unevaluated. Because isoflurane has cerebrovasodilatory properties, it may augment CBF during ischemia and thus significantly decrease the severity of the ischemic insult. Furthermore, if isoflurane attenuates the severity of delayed postischemic hypoperfusion,⁸⁻¹⁰ neuronal recovery during reperfusion may be improved and a mechanistic explanation for the beneficial effects of isoflurane on outcome may be found.

This study compared regional CBF before, during, and after near-complete ischemia in rats anesthetized with either isoflurane or fentanyl-N₂O. We hypothesized that (1) anesthetics differentially influence periischemic CBF, thereby altering the severity of the ischemic insult, and (2) that ganglionic blockade achieved with trimethaphan at onset of ischemia in rats anesthetized with isoflurane has an effect on CBF during ischemia or postischemic reperfusion.

Methods

The following study was approved by the Duke University Animal Care and Use Committee. Male Sprague-Dawley rats (8–10 weeks old; Harlan Sprague-Dawley, Indianapolis, IN) underwent fasting for 12–16 h but had free access to water. The animals were then anesthetized with 5% isoflurane in oxygen. After orotracheal intubation, the lungs were mechanically ventilated (30% O₂-balance N₂). The inspired isoflurane concentration was reduced to 2.0–2.5%. Surgery was performed using the aseptic technique, and all surgical fields were infiltrated with 0.1–0.2 ml lidocaine, 1%. Cannulation was performed on the tail artery and used for blood pressure monitoring and arterial blood sampling. In addition, catheters were placed *via* surgical incision in the right femoral vein and artery for isotope infusion and collection of timed arterial blood samples, respectively. *Via* a

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ventral neck incision, cannulation was performed on the right jugular vein for drug infusion and blood withdrawal. The common carotid arteries were encircled with suture. The vagus nerves and cervical sympathetic plexus were left intact. Muscle paralysis was provided by a 1 mg intravenous bolus of succinylcholine, repeated as necessary to allow control of ventilation during ischemia. Pilot studies had been performed to ensure that rats would not exhibit an escape response in the absence of succinylcholine, given the respective anesthetic regimens. Bilateral cortical electroencephalographic (EEG) responses were continuously monitored during the experiment from active subdermal electrodes positioned over the parietal cortex bilaterally, a reference electrode placed on the nasion, and a ground lead positioned in the tail.

A 22-gauge needle thermistor (Model 524; YSI Co., Yellow Springs, OH) was percutaneously placed adjacent to the skull beneath the temporalis muscle, and pericranial temperature was servoregulated at $37.5 \pm 0.1^\circ\text{C}$ by surface heating or cooling. Heparin, 50 IU, was administered intravenously. After surgical preparation, a 20-min interval was allowed for physiologic stabilization.

For determination of preischemic CBF (just before ischemia), rats were randomly assigned to one of two groups:

1. fentanyl- N_2O or isoflurane ($n = 9$): Isoflurane was discontinued. An intravenous infusion of fentanyl was begun ($10 \mu\text{g}/\text{kg}$ bolus followed by $25 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). The inspiratory gas mixture was changed to 30% O_2 -70% N_2O .
2. Isoflurane ($n = 11$). Isoflurane, 1.4%, was inspired in 30% O_2 -balance N_2 .

For determination of inraischemic CBF (at 8 min after onset of ischemia), rats were randomly assigned to one of three groups:

1. Fentanyl- N_2O ($n = 5$). Isoflurane was discontinued. An intravenous infusion of fentanyl was begun ($10 \mu\text{g}/\text{kg}$ bolus followed by $25 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). The inspiratory gas mixture was changed to 30% O_2 -70% N_2O .
2. Isoflurane ($n = 5$). Isoflurane, 1.4%, was inspired in 30% O_2 -balance N_2 .
3. Isoflurane + trimethaphan ($n = 5$). Isoflurane, 1.4%, was inspired in 30% O_2 -balance N_2 . Trimethaphan (2.5 mg intravenously) was administered at the onset of ischemia.

For determination of postischemic CBF (30 min after onset of reperfusion), rats were randomly assigned to one of three groups:

1. Fentanyl- N_2O ($n = 9$). Isoflurane was discontinued. An intravenous infusion of fentanyl was begun ($10 \mu\text{g}/\text{kg}$ bolus followed by $25 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). The inspiratory gas mixture was changed to 30% O_2 -70% N_2O .

2. Isoflurane ($n = 8$). Isoflurane, 1.4%, was inspired in 30% O_2 -balance N_2 ;
3. Isoflurane + trimethaphan ($n = 8$). Isoflurane, 1.4%, was inspired in 30% O_2 -balance N_2 . Trimethaphan (2.5 mg intravenously) was administered at the onset of ischemia.

A 30-min interval was allowed to establish the respective anesthetic states. Minute ventilation was adjusted to maintain partial pressure of arterial carbon dioxide (Paco_2) within 36–42 mmHg.

Ischemia was induced as follows. Mean arterial pressure (MAP) was reduced by venous blood withdrawal to 30 mmHg, followed by bilateral carotid occlusion using temporary aneurysm clips.^{6,11} In animals that were used for measurements of postischemic CBF, ischemia persisted for 10 min. To discontinue ischemia, shed blood was reinfused, and the aneurysm clips were removed from the carotid arteries. NaHCO_3 (0.3 mEq intravenously) was administered to counteract systemic acidosis.

At the respective CBF measurement intervals, 100 $\mu\text{Ci}/\text{kg}$ of 4-iodo-n-methyl- ^{14}C]antipyrine (^{14}C -IAP, specific activity 60 mCi/mmol, American Radiolabeled Chemicals, Inc., St. Louis, MO) was infused intravenously over 60 s in a 60-step ramp ($10 \mu\text{l}/\text{min}$ – $610 \mu\text{l}/\text{min}$) to produce an increasing ^{14}C -IAP arterial concentration. Twelve timed, 20- μl arterial blood samples (one sample every 5 s) were collected during ^{14}C -IAP infusion for determination of arterial ^{14}C activity. Immediately after the last blood sample and at completion of isotope infusion, all animals were decapitated and brains were rapidly frozen in 2-methylbutane (-40°C). Brain sections and blood samples were prepared for autoradiographic analysis according to previously described methods.^{12–14} Autoradiographic images of six anatomically standardized brain sections were analyzed by an observer blind to experimental group. Regional CBF values were determined from at least three sections, and values for each of the six different regions for each animal were averaged. In addition, autoradiographic density histograms were compiled for each region of interest to determine the percentage cross-sectional area within each region where flow was less than $15 \text{ ml } 100 \text{ gm}^{-1} \cdot \text{min}^{-1}$.

Statistical Analysis

In separate analysis for each measurement interval (i.e., before, during, and after ischemia) physiologic and regional CBF values were compared among the treatment groups by one-way analysis of variance. *Post hoc* test for between group difference was performed using the Fisher protected least-significant difference test when indicated by a significant F ratio. Statistical significance was assumed when $P < 0.05$. Values are reported as mean \pm SD. Statistical evaluations were performed using StatView 5.0 (SAS Institute Inc., Cary, NC).

Table 1. Physiologic Values

	Fentanyl-N ₂ O	Isoflurane	Isoflurane + Trimethaphan
Presurgical body weight (gm)	293 ± 15	293 ± 12	293 ± 10
10 min preischemia			
MAP (mmHg)	133 ± 13	97 ± 8*	96 ± 8*
pH _a	7.35 ± 0.02	7.34 ± 0.04	7.34 ± 0.03
Paco ₂ (mmHg)	38 ± 2	39 ± 2	37 ± 2
Pao ₂ (mmHg)	146 ± 18	140 ± 17	148 ± 19
Glucose (mg/dl)	132 ± 14	137 ± 9	148 ± 19
Hematocrit (%)	43 ± 1	42 ± 1	43 ± 1
Temperature	37.4 ± 0.1	37.4 ± 0.1	37.4 ± 0.1
Shed blood volume (ml)	8.7 ± 1.5†	6.9 ± 1.5†	4.5 ± 1.4
10 min postischemia			
MAP (mmHg)	119 ± 9	103 ± 8*	114 ± 8
pH _a	7.30 ± 0.05	7.33 ± 0.02	7.33 ± 0.03
Paco ₂ (mmHg)	39 ± 2	39 ± 3	39 ± 2
Pao ₂ (mmHg)	143 ± 15	145 ± 6	152 ± 12
Glucose (mg/dl)	108 ± 23	134 ± 13	135 ± 18
Hematocrit (%)	42 ± 1	41 ± 1	41 ± 1
Pao ₂ (mmHg)	143 ± 25	119 ± 19	137 ± 17
30 min postischemia			
MAP (mmHg)	109 ± 11	91 ± 7*	101 ± 13

All values are mean ± SD.

* $P \leq 0.04$.

† $P \leq 0.01$.

MAP = mean arterial pressure; N₂O = nitrous oxide; Paco₂ = arterial carbon dioxide partial pressure; Pao₂ = arterial oxygen partial pressure; pH_a = arterial pH; Sao₂ = arterial hemoglobin oxygen saturation.

Results

Physiologic values are presented in table 1. Pre- and postischemic MAP was less in rats anesthetized with isoflurane than in those administered fentanyl-N₂O ($P \leq 0.04$). During the ischemic interval, EEG isoelectricity was present in all animals that were included in later analysis. Five animals (three in the isoflurane group, two in the fentanyl-N₂O group) were excluded because the criterion of intranscortical EEG isoelectricity was not reached. Pericranial temperature was controlled in all animals at $37.5 \pm 0.2^\circ\text{C}$.

Regional CBF values for six selected regions are presented in figure 1. Preischemic CBF did not differ between isoflurane and fentanyl-N₂O anesthetized animals in any region.

During ischemia, no difference in blood flow was detected among groups in any region. The ischemic insult caused uniform blood flow reduction in the caudoputamen, cortex, and hippocampus to 5% or less of preischemic values. Thalamic blood flow was reduced to 10% or less of preischemic values. Blood flow in the cerebellum and pontine nucleus was attenuated but preserved well above critical flow levels. In these caudal regions, less than 5% of tissue had blood flow values less than $15 \text{ ml} \cdot 100 \text{ gm}^{-1} \cdot \text{min}^{-1}$.

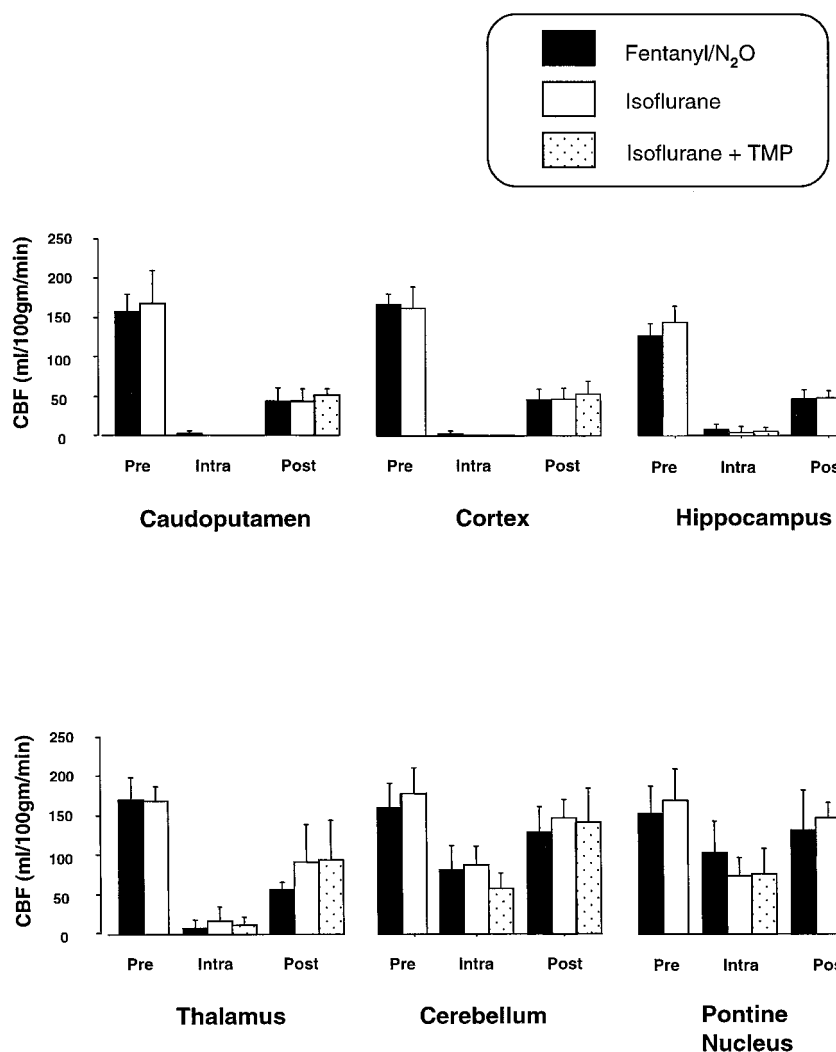
At 30 min after onset of reperfusion, there were no differences among groups for mean blood flow values in the caudoputamen, cortex, hippocampus, or thalamus. Flow remained reduced by 50–65% relative to preischemic values in all three groups. In all groups, less than

15% of cross-sectional area in the caudoputamen, cortex, hippocampus, and thalamus had flow less than $15 \text{ ml} \cdot 100 \text{ gm}^{-1} \cdot \text{min}^{-1}$. However, differences between groups for percent cross-sectional area with flow less than $15 \text{ ml} \cdot 100 \text{ gm}^{-1} \cdot \text{min}^{-1}$ were observed in cortex (fentanyl-N₂O = 15% vs. isoflurane + trimethaphan = 3%; $P = 0.01$; and isoflurane = 11% vs. isoflurane + trimethaphan = 3%; $P = 0.03$) and hippocampus (fentanyl-N₂O = 11% vs. isoflurane + trimethaphan = 6%; $P = 0.02$). Blood flow in the brain stem and cerebellum returned to greater than 80% of preischemic values without differences among groups.

Discussion

Isoflurane is a frequently used volatile anesthetic with cerebrovasodilatory effects and has repeatedly been shown to improve outcome in laboratory models of cerebral ischemia.^{1–4} In a recent study, isoflurane improved histologic outcome after severe forebrain ischemia when compared with fentanyl-N₂O anesthesia, an effect that was completely reversed by sympathetic ganglionic blockade with trimethaphan.⁵ The purpose of this investigation was to evaluate whether beneficial effects of isoflurane after near-complete ischemia in rats could be attributed to its effects on CBF and whether the presence of trimethaphan would alter CBF during or after ischemia. The results of this study show no CBF differences between anesthetic groups in any structure evaluated before or during ischemia. Trimethaphan administered to animals anesthetized with isoflurane did

Fig. 1. Regional cerebral blood flow (CBF) values for rats 10 min before (Pre), during (Intra), or 30 min after ischemia (Post). Rats were anesthetized with either fentanyl-N₂O or isoflurane (with or without 2.5mg intravenous trimethaphan [TMP] at onset of ischemia for Intra and Post measurements). No differences were present among groups. Bilateral carotid occlusion and reduction of mean arterial pressure to 30 mmHg caused a marked blood flow reduction to 5% or less of baseline values ($P < 0.001$) in the cortex, caudoputamen, hippocampus, and thalamus, whereas flow in the brain stem and cerebellum was preserved. Reperfusion at 30 min was associated with a 35–50% restoration of flow to baseline values in the ischemic structures.



not affect intraischemic CBF. Although pronounced delayed postischemic hypoperfusion was observed in all groups, neither anesthetics nor trimethaphan were found to significantly alter this event.

Anesthetic conditions and doses chosen for this study were based on two recent studies that showed a neuroprotective effect of 1.4% isoflurane when compared with fentanyl-N₂O during near-complete forebrain ischemia.^{4,5} Known physiologic determinants of CBF, such as P_{aCO_2} , partial pressure of arterial oxygen (P_{aO_2}), and temperature, were kept within physiologic ranges in all animals. Rats anesthetized with isoflurane showed a consistent pattern of lower preischemic and postischemic MAP when compared with rats anesthetized with fentanyl-N₂O. However, substantially greater differences in MAP are necessary to alter histologic outcome in this model.¹⁵ During ischemia, MAP was held at 30 mmHg in all groups. This, in combination with bilateral carotid occlusion, resulted in consistent EEG isoelectricity in all animals included in the study. Miura *et al.*⁴ have also shown that the near-complete ischemic injury, as used in the current experiment, is uniformly severe among sub-

jects in different anesthetic groups. Cortical and hippocampal EEG recordings in that study were homogeneous with respect to presence of isoelectricity, and all animals underwent ischemic depolarization. However, the CBF thresholds for EEG isoelectricity might be significantly higher than those necessary to elicit depletion of high-energy brain phosphates, disturbances of cellular ion homeostasis, and neuronal cell death.^{11,16,17}

The preischemic CBF values in rats anesthetized with minimum alveolar concentration (MAC) isoflurane are in agreement with previous reports.^{18–21} Preischemic CBF in rats anesthetized with isoflurane did not differ significantly from those administered fentanyl-N₂O. As expected from previous studies of near-complete forebrain ischemia in rats,^{6,7} intraischemic CBF values in the hippocampus, cortex, and caudoputamen were reduced to 5% or less of baseline values in all three groups. Choice of anesthetic or presence of trimethaphan in rats anesthetized with isoflurane did not have a significant impact on this parameter. Accordingly, we can refute the hypothesis that isoflurane has an impact on intraischemic CBF that alters the severity of the ischemic insult.

Although severe postischemic hypoperfusion was observed at 30 min after the end of ischemia, there was also no effect of either anesthetics or trimethaphan on the absolute severity of this condition. The only differences detected were in the amount of tissue with postischemic CBF less than $15 \text{ ml} \cdot 100 \text{ gm}^{-1} \cdot \text{min}^{-1}$ between fentanyl- N_2O and isoflurane-plus-trimethaphan-treated animals (cortex and hippocampus) and isoflurane- and isoflurane-plus-trimethaphan-treated animals (hippocampus), but not between isoflurane- and fentanyl- N_2O -treated rats. However, because isoflurane-anesthetized animals previously had better histologic outcomes than fentanyl- N_2O -treated and isoflurane-plus-trimethaphan-treated animals (with no difference between the latter two groups),⁵ we conclude that these minor flow differences do not impact histologic outcome significantly.

The syndrome of delayed postischemic hypoperfusion characterizes a phase of increased vascular resistance after an initial phase of postischemic reactive hyperemia.²² Postischemic hypoperfusion has been described in rat models of focal,²³ incomplete,²⁴ and complete global²⁵ ischemia, but the underlying mechanisms are understood incompletely. Possible explanations include an imbalance of preserved autoregulation and diminished carbon dioxide reactivity, resulting in arteriolar vasoconstriction²²; expression of endothelial adhesion molecules, with consequent aggregation of polymorphonuclear leukocytes²⁶ and formation of endothelial microvilli²⁷; and increased blood viscosity or microvascular compression by extracellular edema and glial swelling.²⁸ The magnitude of hypoperfusion observed in the current study is comparable with previous investigations. Neither vasodilative effects of isoflurane nor sympatholytic properties of trimethaphan were able to attenuate this postischemic disturbance in CBF. The latter observation is in agreement with Blomqvist *et al.*,²⁵ who reported that lesioning of the locus coeruleus does not influence postischemic hypoperfusion in rats after complete global ischemia.

In summary, this study compared regional CBF before, during, and after near-complete forebrain ischemia in rats anesthetized with either isoflurane (with and without trimethaphan) or fentanyl- N_2O . The results of this study present convincing evidence that previously reported neuroprotective effects of isoflurane and reversal of this protection by trimethaphan cannot be explained by differential vasodilative effects of the anesthetics or trimethaphan during severe forebrain ischemia or postischemic delayed hypoperfusion. Mechanisms other than effects on CBF are responsible for beneficial effects of isoflurane in this model.

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