Anesthesiology 2003; 99:1145–51

Severe Hypotension Is Not Essential for Isoflurane Neuroprotection against Forebrain Ischemia in Mice

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Background: **Volatile anesthetics provide protection in experimental models of global cerebral ischemia. To date, all models evaluated have included profound systemic arterial hypotension as a component of the ischemic insult. This study was designed to determine if isoflurane protection persists in a global insult devoid of hypotension.**

Methods: **C57BL/6J mice having a high incidence of posterior communicating artery atresia were anesthetized with isoflurane (1.2%) or fentanyl/N_2O and subjected to bilateral carotid artery occlusion for 15 min or 20 min with normotension (80–110 mmHg mean arterial pressure) or for 10 min with hypotension (35 mmHg mean arterial pressure). Three days later, neurologic function and histologic damage were assessed. Other mice underwent measurement of intraischemic cerebral blood flow (4-iodo-N-methyl-[^{14}C]antipyrine autoradiography) or plasma norepinephrine.**

Results: **Isoflurane reduced the percentage of hippocampal CA1 dead neurons (*e.g.*, 10 min bilateral carotid occlusion + hypotension: 43 ± 18 (isoflurane) *vs.* 67 ± 20 (fentanyl/N_2O), $P = 0.003$; 20 min bilateral carotid occlusion + normotension: 49 ± 27 (isoflurane) *vs.* 71 ± 22 (fentanyl/N_2O), $P = 0.003$). Isoflurane also reduced CA3 damage and improved neurologic function under all conditions. Intraischemic forebrain blood flow was similar during bilateral carotid occlusion plus normotension for the two anesthetic states. Plasma norepinephrine values were greater when hypotension was added to the ischemic insult.**

Conclusions: **Isoflurane resulted in improved neurologic function and reduced histologic damage regardless of the presence or absence of systemic hypotension during the ischemic insult. This indicates that beneficial effects of isoflurane are most likely attributable to direct effects at the neuronal level as opposed to indirect effects resulting from interactions with profound hypotension.**

ISOFLURANE has been shown to reduce brain damage in a variety of laboratory cerebral ischemia models.[1–5] Although protection against focal ischemia could be predicted because of the substantial reduction in cerebral metabolic rate for oxygen consumption caused by isoflurane,[6] it came as some surprise that isoflurane is also protective against near-complete global ischemia.[2,3] The mechanism for this protection is not well defined. Severe forebrain ischemia causes, in and of itself, abrupt and complete cessation of electroencephalographic activity.[7] If the mechanism of isoflurane protection is reduction in synaptic neurotransmission as manifested by reduction in electroencephalographic activity, there would be no opportunity for isoflurane to protect against a severe forebrain ischemic insult sufficient to cause electroencephalographic isoelectricity.

One commonality to all studies that have identified isoflurane protection against global ischemia is the concomitant use of profound hypotension during the ischemic insult. Both the unilateral and bilateral carotid artery occlusion (BCAO) models used in rats require reduction of mean arterial pressure (MAP) to approximately 35 mmHg to reduce cerebral blood flow (CBF) below ischemic threshold values and cause consistent histologic damage.[1–3,8] Isoflurane protection has also been demonstrated in a canine model of cardiac arrest, where MAP was decreased even further.[5]

Profound hypotension results in marked increases in circulating catecholamine concentrations, which have been postulated to modulate ischemic brain injury.[9,10] Isoflurane has been shown to attenuate some of these responses.[3,11,12] Therefore, it can be argued that isoflurane neuroprotection may be mediated by an effect on responses to hypotension as opposed to isoflurane having a direct neuronal mechanism of action.

The C57BL/6J mouse is known to have an incomplete circle of Willis with a high frequency of posterior communicating artery atresia.[13–16] Like the Mongolian gerbil,[17] this results in segregated anterior and posterior circulations. In these species, occlusion of the carotid arteries in the absence of hypotension is sufficient to generate a dense ischemic insult with resultant neuronal necrosis in selectively vulnerable regions of the forebrain.[15] We exploited this anatomic C57BL/6J variant to test whether isoflurane neuroprotection against severe forebrain ischemia would persist in the absence of concomitant arterial hypotension.

Methods

This study was approved by the Duke University Animal Care and Use Committee. Male C57BL/6J mice (8–10 weeks of age; Jackson Laboratories, Bar Harbor, ME) were overnight fasted from food but allowed free access to water. Anesthesia was induced with 3% isoflurane in a mixture of 50% oxygen/50% nitrogen. The tracheas were intubated (20-gauge Insyte-W intravenous catheter; Becton-Dickinson, Sandy, UT), and the lungs were me-

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Received from the Multidisciplinary Neuroprotection Laboratories, Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina. Submitted for publication July 8, 2003. Accepted for publication July 22, 2003. Supported by grant RO1 GM/NS67139 from the National Institutes of Health, Bethesda, Maryland, and by postdoctoral stipends provided by the Fundacao de Amparo a Pesquisa do Estado de São Paulo, Brazil (to Dr. Homi).

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chanically ventilated (tidal volume = 0.5 ml, respiratory rate = 100 breaths/min). The isoflurane concentration was reduced to 1.2%. A needle thermistor was placed beneath the right temporalis muscle adjacent to the skull. Pericranial temperature was monitored and servoregulated with a surface heating/cooling system to a target of 37.0°C. The right femoral artery was cannulated (PE10 catheter; Becton Dickinson, Sparkes, MD) to monitor MAP and to collect arterial blood samples. The left femoral vein was cannulated with a PE10 catheter to allow continuous infusion of fentanyl or saline. *Via* a ventral neck incision, both common carotid arteries were identified, carefully separated from the vagus nerves, and encircled with silk suture. The right external jugular vein was cannulated with a PE10 catheter.

Three experiments were performed concurrently. For each experiment, 15–20 mice were studied per group.

In experiment 1, animals were randomly assigned to one of two groups:

- isoflurane (1.2%); BCAO (normotension) for 15 min
- fentanyl/N_2O; BCAO (normotension) for 15 min

In experiment 2, mice were randomly assigned to one of two groups:

- isoflurane (1.2%); BCAO (normotension) for 20 min
- fentanyl/N_2O; BCAO (normotension) for 20 min

In experiment 3, mice were randomly assigned to one of two groups:

- isoflurane (1.2%); BCAO (hypotension; MAP = 35 mmHg) for 10 min
- fentanyl/N_2O; BCAO (hypotension; MAP = 35 mmHg) for 10 min

The isoflurane groups were anesthetized with 1.2% isoflurane in 30% O_2/balance N_2. In the fentanyl/N_2O groups, isoflurane was discontinued after surgical preparation and anesthesia was maintained with intravenous fentanyl (10-μg/kg bolus followed by 25 $\mu g^{-1} \cdot kg^{-1} \cdot h^{-1}$) and 70% N_2O/30% O_2. All animals received one intravenous dose of vecuronium (0.1 mg/kg) before the onset of ischemia. Pilot studies were performed, in the absence of vecuronium, to ensure that mice would not exhibit an escape response during either anesthetic regimen. To stabilize the anesthetic state in each group, a period of 30 min was allowed.

Two forms of transient forebrain ischemia were examined. In experiments 1 and 2, forebrain ischemia was induced by bilateral occlusion of the common carotid arteries. The carotid arteries were occluded with aneurysm clips for 15 min (experiment 1) or 20 min (experiment 2). MAP was not manipulated. Reperfusion was achieved by removing the aneurysm clips.

In experiment 3, ischemia was induced by withdrawal of blood (0.6–0.9 ml) from the jugular vein to reduce MAP to 35 mmHg. Both common carotid arteries were occluded with aneurysm clips for 10 min. Maintenance of MAP at 35 mmHg was achieved by further withdrawal of blood as required. Reperfusion was achieved by removal of the clips and rapid infusion of withdrawn blood.

To counteract systemic acidosis, $NaHCO_3$ (8.4%) was given intravenously (15 μl) in all groups. Anesthetic agents were continued after reperfusion for 30 min. The vascular catheters were removed. The wounds were infiltrated with 1% lidocaine and closed with suture. Mice were allowed to recover in a humidified oxygen-enriched environment (0.5 fraction of inspired oxygen). Rectal temperature was maintained at 37.0°C using a heat lamp. On recovery of spontaneous ventilation and the righting reflex, the tracheas were extubated. The mice were then returned to their cages with free access to water and food.

Three days after the ischemic insult, mice were subjected to a series of neurologic tests designed to detect motor deficits in the rat,[18] which have subsequently been modified for the mouse.[19] Briefly, the mice were first placed on a 10- × 20-cm screen (grid size 0.2- × 0.2-cm) that could be rotated from 0 to 90 degrees. The duration of time that the mouse could hold onto the screen after being rotated from horizontal (0°) to vertical (90°) was recorded to a maximum of 15 s. Next, the amount of time that the mouse could remain balanced on a horizontal wooden rod was recorded up to 30 s. Lastly, the animal underwent a prehensile traction test by timing the period that the mouse could cling to a horizontal rope (maximum = 5 s). From these three tests, a maximal score of 9 (normal function) and a minimal score of 0 could be obtained.

Mice were then anesthetized with 3% isoflurane, the trachea was intubated, and the lungs were mechanically ventilated. Transcardiac arterial perfusion was performed initially with saline (30 ml) and then with 10% formalin (30 ml). On the following day, the brains were removed and stored in 10% formalin until paraffin embedding. The brains were then cut into 5-μm thick sections and stained with acid fuchsin–celestine blue.[20] Pyramidal neuronal injury was evaluated in the CA1 and CA3 sectors of the dorsal hippocampus (at bregma −2.8 mm) by an investigator blinded to group assignment using light microscopy (three different random high-power fields per sector, ×400 magnification). Nonviable neurons were considered to be those having a reddish hue. Viable neurons were considered to be those with a blue hue and also having an intact plasma membrane and visible nucleus. Viable and nonviable neurons were manually counted, and the percentage of nonviable neurons was calculated (percentage of dead neurons). At the level where the septal nuclei were widest, damage in the neocortex and caudoputamen was graded (crude damage index) on a 0–3 scale (0 = no damaged neurons, 1 = 1–30% neurons damaged, 2 = 30–60% neurons

damaged, 3 = more than 60% neurons damaged).[21] Values from the hemisphere with the worst damage were used for the final analysis.

In experiment 4, CBF was measured during the ischemic insult. Mice were randomly assigned to one of four groups:

- isoflurane (1.2%) + BCAO (normotension)
- fentanyl/N_2O + BCAO (normotension)
- isoflurane (1.2%) sham surgery, no ischemia
- fentanyl/N_2O sham surgery, no ischemia

These animals underwent identical anesthetic and surgical preparation procedures as described for experiments 1–3. In addition, catheters standardized for length and volume were placed in the left femoral artery and left jugular vein. Using previously described methods[22] at 10 min after the onset of ischemia (after clamping both common carotid arteries in the BCAO groups or an equivalent interval in shams), 5 μCi 4-iodo-N-methyl-[^{14}C]antipyrine in 50 μl saline (^{14}C-IAP, specific activity of 55.4 mCi/mM; American Radiolabeled Chemicals, St. Louis, MO) was infused intravenously over 60 s in a 60-step ramp (0.1–203 μl/min) so as to produce an increasing ^{14}C-IAP arterial concentration. During the infusion, 12 timed 10-μl arterial blood samples were collected for later determination of arterial ^{14}C activity. Samples were collected every 5 s. Blood samples were placed on filter paper and dried for 24 h. Samples were then eluted for 24 h in 1 ml saline and 9 ml liquid scintillation cocktail (CytoScint; ICN, Costa Mesa, CA). After timed blood withdrawal, the mice were rapidly decapitated and the brains were frozen in 2-methylbutane at −20°C within 30 s. Radioactivity was determined *via* liquid scintillation counting using an external quench correction.

Frozen brains were coronally sectioned (20-μm thick) at −16°C. Quadruplicate sections were taken at anatomic levels identical to those used for histologic analysis in the outcome studies (in addition, a section was taken through the brainstem to sample flow in the posterior circulation), mounted on glass slides, dried for 5 min on a hot plate (36°C), and exposed to autoradiographic film (BioMax MR; Eastman Kodak, Rochester, NY) for 3 days along with [^{14}C] methylmethacrylate standards ranging from 0 to 35.0 nCi/mg.

Images from each anatomic level were scanned by a digital camera and stored as a 1280 × 960 matrix of calibrated pixel units (14 μm × 15 μm). Digital optical densities values derived from the autoradiographic images and ^{14}C standards, and timed arterial blood ^{14}C activity values were entered into an image analyzer (MCID-M2, version 3.0, revision 1.2; Imaging Research, St. Catherines, Ontario, Canada). Radioactivity values were converted to CBF values with $\lambda = 0.8$.[23] An observer blinded to experimental group outlined anatomic regions of interest corresponding to the regions where histologic damage was assessed in the outcome studies.

Table 1. Physiologic Values for C57BL/6J Mice Subjected to 15 Min of Bilateral Carotid Artery Occlusion in the Absence of Induced Hypotension (Experiment 1)

	Isoflurane	Fentanyl/N_2O
n	20	20
Body weight		
Before ischemia, g	25 ± 2	25 ± 1
3 days after ischemia, g	25 ± 2	24 ± 2
30 min before ischemia		
MAP, mmHg	76 ± 4	79 ± 5
pHa	7.26 ± 0.04	7.25 ± 0.06
$Paco_2$, mmHg	36 ± 4	35 ± 5
Pao_2, mmHg	208 ± 24	210 ± 17
During ischemia		
MAP, mmHg*	87 ± 10	99 ± 8
Pericranial temperature °C	37.0 ± 0.1	37.0 ± 0.1
10 min after ischemia		
MAP, mmHg	77 ± 9	84 ± 9

Values are mean ± SD.

* Significant difference between groups ($P < 0.05$).

MAP = mean arterial blood pressure; $Paco_2$ = arterial carbon dioxide partial pressure; Pao_2 = arterial oxygen partial pressure; pHa = arterial pH.

In experiment 5, plasma norepinephrine concentrations were measured as a function of both model and anesthetic state. Mice were surgically prepared for ischemia and anesthetized with either isoflurane or fentanyl/N_2O as for experiments 1–3. Five min after onset of ischemia (BCAO with or without hypotension), jugular venous blood (0.5 ml) was withdrawn and placed into a 1-ml vial containing 50 μl ethylenediaminetetraacetic acid (16 mg/ml). After centrifugation, 300 μl plasma was separated and stored at −80°C. Norepinephrine was measured by enzyme-linked immunosorbent assay (CatCombi ELISA RE 592 42; IBL Immuno-Biologic Laboratories, Hamburg, Germany) using known norepinephrine standards within the range of 0–500 ng/ml.

Each of the three outcome studies was considered to be an independent event for statistical analysis. The Student *t* test was used to compare parametric values, including physiologic variables and percentage of dead neurons in the hippocampal CA1 and CA3 sectors. Nonparametric values (crude damage index in neocortex and caudoputamen and total motor score) were compared by the Mann–Whitney U statistic. In the CBF study, regional CBF and physiologic values were compared between groups by one-way ANOVA. When indicated by a significant *F* ratio, *post hoc* testing was performed with the Scheffé test. In the plasma norepinephrine study, values were compared by two-way ANOVA (model × anesthetic). Statistical significant was assumed when $P < 0.05$. Parametric values are reported as mean ± SD. Nonparametric values are reported as median ± interquartile range.

Results

Physiologic values for experiments 1–3 are presented in tables 1–3, respectively. There were no differences

Table 2. Physiologic Values for C57BL/6J Mice Subjected to 20 Min of Bilateral Carotid Artery Occlusion in the Absence of Induced Hypotension (Experiment 2)

	Isoflurane	Fentanyl/N_2O
n	20	20
Body weight		
Before ischemia, g	23 ± 2	24 ± 2
3 days after ischemia, g	23 ± 2	23 ± 3
30 min before ischemia		
MAP, mmHg	75 ± 4	79 ± 5
pHa	7.26 ± 0.06	7.25 ± 0.04
$Paco_2$, mmHg	33 ± 5	34 ± 3
Pao_2, mmHg	225 ± 15	222 ± 17
During ischemia		
MAP, mmHg*	86 ± 7	102 ± 10
Pericranial temperature, °C	37.0 ± 0.1	37.0 ± 0.1
10 min after ischemia		
MAP, mmHg	78 ± 6	82 ± 9

Values are mean ± SD.

* Significant difference between groups ($P < 0.05$).

MAP = mean arterial blood pressure; $Paco_2$ = arterial carbon dioxide partial pressure; Pao_2 = arterial oxygen partial pressure; pHa = arterial pH.

between groups in any experiment with one exception. In both experiments performed without induced hypotension (experiments 1 and 2), intraischemic MAP was greater in the fentanyl/N_2O *versus* isoflurane groups.

Neurologic scores recorded 3 days after ischemia are reported in table 4. For all three outcome experiments (experiments 1–3), function was better in the isoflurane *versus* fentanyl/N_2O groups.

Table 5 provides the histologic data for experiments 1–3. In experiment 1 (15 min BCAO + normotension), animals treated with isoflurane had less damage in three of four regions examined (hippocampal CA1 and CA3 and caudoputamen). In experiment 2 (20 min of BCAO + normotension), histologic damage was less severe in the hippocampal CA1 and CA3 and neocortex in the isoflurane group. In experiment 3 (10 min of BCAO + hypotension), isoflurane-anesthetized mice had reduced damage in hippocampal CA1 and CA3, but there was no difference between anesthetic groups for damage in the caudoputamen or neocortex.

Table 3. Physiologic Values for Mice Subjected to 10 Min of Bilateral Carotid Artery Occlusion and a Target Intraischemic MAP of 35 mmHg (Experiment 3)

	Isoflurane	Fentanyl/N_2O
n	15	15
Body weight		
Before ischemia, g	24 ± 1	25 ± 2
3 days after ischemia, g	24 ± 2	24 ± 3
30 min before ischemia		
MAP, mmHg	75 ± 3	76 ± 4
pHa	7.24 ± 0.05	7.26 ± 0.05
$Paco_2$, mmHg	35 ± 5	35 ± 5
Pao_2, mmHg	221 ± 10	208 ± 25
During ischemia		
MAP, mmHg	35 ± 1	35 ± 1
Pericranial temperature, °C	37.0 ± 0.1	37.0 ± 0.1
10 min after ischemia		
MAP, mmHg	74 ± 9	82 ± 11

Values are mean ± SD. There were no differences between groups.

MAP = mean arterial blood pressure; $Paco_2$ = arterial carbon dioxide partial pressure; Pao_2 = arterial oxygen partial pressure; pHa = arterial pH.

Table 4. Total Motor Scores Recorded 3 Days after Ischemia for Experiments 1–3

	Isoflurane	Fentanyl/N_2O	*P* value
Experiment 1 (15 min BCAO/ normotension)	8 ± 2	5 ± 6	0.008
Experiment 2 (20 min BCAO/ normotension)	8 ± 1	5 ± 4	0.001
Experiment 3 (10 min BCAO/ hypotension)	7 ± 4	3 ± 4	0.028

Values are median ± interquartile range. A score of 9 indicates no deficit. n = 15–20 mice per experimental group.

BCAO = bilateral carotid artery occlusion; MAP, mean arterial pressure; hypotension = venous exsanguination as required to maintain. MAP at 35 mmHg throughout 10 min of BCAO; normotension = MAP of 80–110 mmHg.

For experiment 4 (CBF analysis), physiologic values were similar to those observed in experiments 1 and 2 and therefore are not reported. Table 6 presents the regional CBF data from experiment 4. Among sham mice subjected to the respective isoflurane or fentanyl/N_2O anesthetic states, there was no difference between groups for blood flow in any region examined (hippocampus, caudoputamen, neocortex, and brainstem). Carotid occlusion with normotension profoundly reduced CBF values in the hippocampus, caudoputamen, and neocortex. Although flow values were near zero in the caudoputamen and neocortex, residual flow was observed with a relatively high coefficient of variation in the hippocampus. There was no difference between anesthetic states in any region. Brainstem blood flow was preserved at normal values in both groups as expected because of patency of the vertebrobasilar system.

For experiment 5, physiologic values were similar to those observed in the other experiments and therefore are not reported. A main effect for model (*i.e.*, BACO with or without hypotension) was observed for plasma norepinephrine concentration ($P = 0.01$). There was no effect of anesthetic ($P = 0.50$), and there was no interaction between factors ($P = 0.30$). Plasma norepinephrine concentrations were greater in mice subjected to BCAO plus hypotension (isoflurane: 206 ± 80 ng/ml, n = 6; fentanyl/N_2O: 159 ± 74 ng/ml, n = 6) *versus* BCAO alone (isoflurane: 105 ± 48 ng/ml, n = 6; fentanyl/N_2O: 115 ± 31 ng/ml, n = 5).

Discussion

This study demonstrates that neurologic and histologic outcome from severe forebrain ischemia can be dependent on the anesthetic present during the ischemic insult. Mice anesthetized with isoflurane had improved

Table 5. Histologic Damage as a Function of Anesthetic Group in Experiments 1–3

	Isoflurane	Fentanyl/N_2O	P value
Experiment 1 (15 min BCAO/normotension)			
CA1	47 ± 21	69 ± 25	0.003
CA3	52 ± 12	69 ± 22	0.006
Caudoputamen	3 ± 1	3 ± 0	0.037
Neocortex	1 ± 1	1 ± 1.5	0.098
Experiment 2 (20 min BCAO/normotension)			
CA1	49 ± 27	71 ± 22	0.003
CA3	55 ± 26	79 ± 20	0.003
Caudoputamen	3 ± 0	3 ± 0	0.152
Neocortex	1 ± 1	1.5 ± 1	0.006
Experiment 3 (10 min BCAO/hypotension)			
CA1	43 ± 18	67 ± 20	0.002
CA3	48 ± 15	66 ± 21	0.002
Caudoputamen	1 ± 1	2 ± 1	0.555
Neocortex	0 ± 1	1 ± 1	0.155

Values for hippocampal CA1 and CA3 are percentages of dead neurons (mean ± SD). For the caudoputamen and neocortex, crude damage index scores (median ± interquartile range) are given (0 = no damage; 3 = > 60% neurons damaged). n = 20 per group for Experiments 1 and 2, n = 15 per group for Experiment 3.

BCAO = bilateral carotid artery occlusion; hypotension = venous exsanguination as required to maintain MAP at 35 mmHg throughout 10 min of BCAO; MAP = mean arterial pressure; normotension = MAP of 80–110 mmHg.

neurologic function and reduced histologic injury compared with those anesthetized with fentanyl/N_2O. These findings were independent of the method by which ischemia was induced. Notably, the improved outcome observed in isoflurane-anesthetized mice was present whether or not induced hypotension was concurrent with the ischemic insult. The differences between groups could not be attributed to differential effects of the anesthetics on intraischemic blood flow. Blood flow reflected a severe diffuse forebrain insult that was of a similar magnitude for the two anesthetic groups. Finally, the BCAO plus hypotension model was found to produce greater plasma concentrations of norepinephrine during ischemia, consistent with a more systemically stressful insult than occurred with BCAO alone. Mice treated with isoflurane had improved outcomes regardless of the presence or absence of profound hypotension. As a result, it can be concluded that the mechanism of action of isoflurane as a neuroprotectant does not require interaction with stress responses to hemodynamic shock. This supports the concept that the neuroprotective effects of isoflurane represent direct effects occurring at the cerebral cellular level.

We believe that our experiment was methodologically sound. Relevant physiologic variables were monitored and controlled with one exception. Plasma glucose was not monitored, because the blood volume of the mouse is so small that blood sampling beyond that required for blood gas analysis would pose a hypovolemic state that may have influenced the overall outcome. All mice were fasted for at least 12 h before ischemia. Similar studies in the rat, where plasma glucose was measured, found no difference between isoflurane and fentanyl/N_2O anesthetic states.[2,3,24] We therefore do not believe that plasma glucose concentrations were a confounding factor in the present study. There was a difference between

Table 6. Regional Cerebral Blood Flow Values (Experiment 4)

	Isoflurane Sham (n = 5)	Fentanyl/N_2O Sham (n = 4)	Isoflurane BCAO (n = 8)	Fentanyl/N_2O BCAO (n = 8)
Hippocampus				
Right	117 ± 33	117 ± 23	6 ± 15	7 ± 17
Left	117 ± 33	115 ± 24	2 ± 2	4 ± 4
Neocortex				
Right	118 ± 35	116 ± 22	0 ± 0	1 ± 1
Left	122 ± 39	121 ± 19	0 ± 0	1 ± 1
Caudoputamen				
Right	115 ± 33	124 ± 36	2 ± 5	1 ± 2
Left	129 ± 39	119 ± 26	0 ± 0	1 ± 1
Brainstem	137 ± 37	125 ± 21	145 ± 32	125 ± 21

Values (ml · 100 g · min) represent mean ± SD.

A main effect ($P < 0.0001$) was present for all regions except the brainstem ($P = 0.61$). In both hemispheres (right and left), for the hippocampus, neocortex, and caudoputamen, there was no difference between isoflurane sham and fentanyl/N_2O sham. Similarly there was no difference between isoflurane BCAO and fentanyl/N_2O BCAO. For each region there was a difference between both BCAO groups and both sham groups ($P < 0.0001$).

BCAO = bilateral carotid artery occlusion with normotension (mean arterial pressure = 80–110 mmHg).

anesthetic groups for intraischemic MAP in experiments 1 and 2, where carotid occlusion was studied in the absence of induced hypotension. Because of this, experiment 4 was performed to ensure that the magnitude of CBF reduction was the same in both anesthetic groups subjected to BCAO. This was confirmed. In fact, MAP was greater in the fentanyl/N_2O group, which, if anything, would be expected to have improved outcome in that group.

The normotensive BCAO model has been previously investigated in the C57BL/6J mouse[16] and has become an accepted model for study of neuroprotective mechanisms of action.[25,26] Carotid occlusion in the absence of induced hypotension was found to provide more variability in the magnitude of CBF reduction than if induced hypotension were added to the ischemic insult.[16] For this reason, longer durations of ischemia are required to cause a similar magnitude of cellular necrosis.[16,25] This explains our experimental design in which the durations of ischemia were 15 min and 20 min in experiments 1 and 2, where an attempt was made to define a dose–response relation between duration of ischemia and outcome. Despite the difference in ischemic duration, a numeric difference in the magnitude of tissue damage was not evident, although the protective effect of isoflurane persisted. We performed pilot studies attempting to generate a 15-min BCAO plus hypotension insult that would allow a more direct comparison between the two models. This duration was abandoned, however, because of a high incidence of acute mortality, presumably caused by extracerebral effects of sustained hemorrhagic shock.

Several studies have attempted to associate catecholamine responses to ischemia with outcome.[9,10] The results of such work have been contradictory and nonconclusive. Catecholamine and corticosterone concentrations in the brain and blood are differentially affected by isoflurane and fentanyl/N_2O during severe forebrain ischemia.[3,11,12] The anesthetic effects in blood and brain seem to be uncoupled, however. More importantly, treatment with chloroethyl-N-ethyl-2-bromobenzylamine before ischemia, which reduces brain norepinephrine concentrations to less than 5% of control, had no effect on outcome in rats subjected to severe forebrain ischemia.[27] These data, in combination with the results of the current study, make it highly unlikely that the mechanism of action of isoflurane protection is attributable to hypotension or hormonal responses to hypotension.

This study evaluated outcome at 3 days after the ischemic insult. This interval has been shown to reflect the peak of CA1 damage in both rats and mice after severe forebrain ischemia within the first week of recovery.[15,28] Nevertheless, it must be noted that there is sufficient evidence in the literature to argue that final outcome was not measured in our experiment. Although studies defining apoptotic responses to ischemia have largely been performed in focal ischemia models,[29] Kawaguchi *et al.*[30] have shown that isoflurane protection, although evident at 2 days after the ischemic insult, had dissipated after 14 days of recovery from middle cerebral artery occlusion. Therefore, the current study did not define outcome with sufficient duration to provide conclusive evidence that the beneficial effects observed for isoflurane are permanent.

This study was designed to examine interactions with responses to hypotension as a mechanism of isoflurane neuroprotective action. That hypothesis can comfortably be rejected. As a result, this experiment did not define the mechanism of action of isoflurane. Both *in vivo* and *in vitro* studies have determined that isoflurane neuroprotection can, at least in part, be attributed to antagonism of the N-methyl-D-aspartate receptor.[31–34] This effect is insufficient to explain fully its mechanism of action. Isoflurane is also known to potentiate the inhibitory actions of γ-amino-butyric acid.[35,36] *In vitro* evidence is currently being sought to support this as a component of isoflurane's protective effects. The current study does not provide evidence to support these mechanisms of action specifically but is consistent with a direct cellular effect of isoflurane.

In summary, isoflurane was found to improve histologic outcome from severe forebrain ischemia in mice subjected to either normotensive or hypotensive BCAO. This effect could not be attributed to effects on intraischemic blood flow. Volatile anesthetics have been shown to improve outcome from ischemic insults in the cat,[37] dog,[5] rat,[1,2] and, now, the mouse. Continued investigation of the appropriate use of this drug and its mechanism of action is warranted.

The authors are grateful for the expert histologic technical assistance provided by Ann D. Brinkhous, Technician, Department of Anesthesiology, Duke University Medical Center, Durham, North Carolina.

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