Sympathetic Ganglionic Blockade Masks Beneficial Effect of Isoflurane on Histologic Outcome from Near-complete Forebrain Ischemia in the Rat

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Background: Isoflurane-anesthetized rats have better outcome from global cerebral ischemia than rats anesthetized with fentanyl and nitrous oxide. The authors wanted to determine whether circulating catecholamine concentrations depend on the anesthetic agent and whether sympathetic ganglionic blockade affects anesthetic-mediated differences in outcome from near-complete forebrain ischemia.

Methods: For two different experiments, normothermic Sprague-Dawley rats that had fasted were assigned to one of four groups and subjected to 10 min of 50 mmHg mean arterial pressure and bilateral carotid occlusion. Rats were anesthetized with 1.4% isoflurane or fentanyl (25 µg · kg⁻¹ · h⁻¹) and 70% nitrous oxide, with or without preischemic trimethaphan (2.5 mg given intravenously). In experiment 1, arterial plasma catecholamine concentrations were measured before, at 2 and 8 min during, and after ischemia (n = 5–8). In experiment 2, animals (n = 15) underwent histologic analysis 5 days after ischemia.

Results: In experiment 1, intras ischemic increases in plasma norepinephrine and epinephrine levels were 28 and 12 times greater in the fentanyl-nitrous oxide group than in the isoflurane group (P < 0.01). Trimethaphan blocked all changes in plasma catecholamine concentrations (P < 0.02). In experiment 2, isoflurane reduced the mean ± SD percentage of dead hippocampal CA1 neurons compared with fentanyl–nitrous oxide (43 ± 22% vs. 87 ± 10%; P < 0.001). Trimethaphan abolished the beneficial effects of isoflurane (91 ± 6%; P < 0.001). Similar observations were made in the cortex.

Conclusions: Isoflurane attenuated the peripheral sympathetic response to ischemia and improved histologic outcome compared with fentanyl and nitrous oxide. This outcome benefit was reversed by sympathetic ganglionic blockade. The beneficial effects of isoflurane may result from a neuroprotective influence of an intermediate sympathetic response that is abolished by trimethaphan. (Key words: Anesthetics; hippocampus; histology.)

ANESTHETICS alter tolerance of the brain to ischemia.¹,² Beneficial effects of the volatile anesthetic isoflurane on outcome from either focal³ or incomplete global ischemia have been shown.⁴,⁵ These observations were supported by a recent laboratory study of severe forebrain ischemia.⁶ Isoflurane-anesthetized rats had better histologic and behavioral outcomes than did rats given ketamine or fentanyl and nitrous oxide (N₂O). Differences in the depth and character of anesthesia and associated influences on the sympathetic state might have accounted for the observed outcome effects of anesthetic agents in that global ischemia model.

Ischemic brain injury activates the sympathetic nervous system, increasing central and peripheral catecholamine concentrations.⁷,⁸ The effect of this stress re-
sponse on outcome from ischemic brain injury is controversial. In a model of severe hemispheric ischemia, Werner et al.9 showed that sympathetic ganglionic blockade, achieved with hexamethonium, improved neurologic outcome. The administration of exogenous catecholamines diminished the beneficial effect of ganglionic blockade and worsened neurologic outcome. Further research supported the conclusion that a reduction of catecholamine concentrations is neuroprotective.10-12 In contrast, Koide et al.13 reported that the use of the ganglionic blocking agent trimethaphan in a model of severe forebrain ischemia increased histologic damage. Co-administration of exogenous catecholamines reversed the effect of trimethaphan and improved histologic outcome. Further investigations supported the finding that increased catecholamine concentrations are neuroprotective.14-16 However, none of these apparently contradictory sets of experiments was obtained during conditions of brain temperature control, which are now known to be important. To evaluate further the effect of circulating catecholamines on ischemic outcome, we tested the following hypotheses: 1) Circulating catecholamine concentrations during ischemia depend on the anesthetic agent and can be modulated by sympathetic ganglionic blockade and 2) sympathetic ganglionic blockade can prevent anesthetic-mediated differences in histologic outcome from a severe forebrain ischemic injury.

Methods

Experiment 1

The following studies were approved by the Duke University Animal Care and Use Committee. Male Sprague-Dawley rats (8–10 weeks old; Harlan Sprague-Dawley, Indianapolis, IN) were fasted for 12–16 h but had free access to water. The animals were anesthetized with 5% isoflurane in oxygen. After orotracheal intubation, the lungs were ventilated mechanically (30% oxygen–balance nitrogen). The inspired isoflurane concentration was reduced to 2 to 2.5%. Surgery was performed using an aseptic technique, and all surgical fields were infiltrated with 1% lidocaine. The tail artery was cannulated and used for blood pressure monitoring and arterial blood sampling. Via a ventral neck incision, the right jugular vein was cannulated with a silicone catheter for drug infusion and blood withdrawal. The common carotid arteries were encircled with sutures. 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 electric activity of the brain was monitored continuously 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 placed percutaneously adjacent to the skull beneath the temporalis muscle, and the pericranial temperature was servoregulated (model 73AAT indicating controller; YSI Co.) at 37.5 ± 0.1°C by surface heating or cooling. Heparin (50 IU) was given intravenously. After surgical preparation, a 20-min interval was allowed for physiologic stabilization.

Rats were assigned randomly to one of four groups:

1. Fentanyl-N2O (n = 8): Isoflurane was discontinued. An intravenous infusion of fentanyl was begun (10 μg/kg bolus followed by 25 μg·kg⁻¹·h⁻¹). The inspiratory gas mixture was changed to 30% oxygen and 70% N2O.
2. Fentanyl-N2O + trimethaphan (n = 5): Isoflurane was discontinued. An intravenous infusion of fentanyl was begun (10 μg/kg bolus followed by 25 μg·kg⁻¹·h⁻¹). The inspiratory gas mixture was changed to 30% oxygen and 70% N2O. Trimethaphan (2.5 mg given intravenously; Arfonad, Hoffman-La Roche, Nutley, NJ) was given at the onset of ischemia.
3. Isoflurane (n = 8): 1.4% isoflurane inspired in 30% oxygen and balance nitrogen.
4. Isoflurane + trimethaphan (n = 5): 1.4% isoflurane inspired in 30% oxygen and balance nitrogen. Trimethaphan (2.5 mg given intravenously) was administered at the onset of ischemia.

An isoflurane concentration of 1.4% was chosen based on a recent study that showed a neuroprotective effect of isoflurane at this dose when compared with fentanyl-N2O during nearly complete forebrain ischemia.9 A 30-min interval was allowed to establish the respective anesthetic states. Ventilation was adjusted to maintain the carbon dioxide tension in arterial blood within 36–42 mmHg.

Mean arterial pressure was reduced by venous blood withdrawal to 30 mmHg, followed by bilateral carotid occlusion using temporary aneurysm clips.17,18 Ischemia
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persisted for 10 min. To discontinue ischemia, shed blood was reinfused and the aneurysm clips were removed from the carotid arteries. To counteract systemic acidosis, 0.3 mEq NaHCO₃ was given intravenously.

Blood samples (200 µl) were taken to determine plasma catecholamines 30 and 2 min before ischemia onset, at 2 and 8 min during ischemia, and at 10, 30 and 120 min after the 10-min ischemic period was discontinued. Sodium metabisulfite and EDTA were added to prevent catecholamine oxidation. Plasma catecholamines were extracted on aluminum and samples were injected into a reverse-phase, high-performance liquid chromatograph equipped with a C-18 column and an electrochemical detector.

Anesthetic agents were continued after ischemia for 120 min. After the conclusion of the study, animals were killed with an overdose of isoflurane.

**Experiment 2**

Male Sprague-Dawley rats (8-10 weeks old) were anesthetized with 50 mg/kg intraperitoneal sodium pentobarbital (50 mg/ml Nembutal; Abbott Laboratories, North Chicago, IL). Each animal was positioned in a stereotactic head frame. Using an aseptic technique, the skin was infiltrated with 1% lidocaine and a midline scalp incision was made. A burr hole was drilled over the right hemisphere, 2 mm lateral to the midline and 3 mm anterior to the bregma. A radiotelemetry thermistor (Brain Probe, model XM-FH-BP; Minimitter Co., Sunriver, OR), accuracy ±0.1°C, was placed on the skull with the tip positioned on the dura. The probe was fixed in place with two cranial screws, and the burr hole was sealed with orthodontic cement. The wound was closed with sutures and the animals were allowed to awaken. Rats were returned to their cages with free access to water and food for recovery.

The thermistor had been calibrated previously (within the range of 35-40°C) in a circulating water bath against a mercury thermometer. This allowed extrapolation of temperatures from calibration points in accordance with the radiofrequency emitted by the probe. Radiofrequency signals from the probe were received (Telemetry Receiver model RA1010; Data Science, St. Paul, MN), digitized, and processed on a personal computer (iDX-35V; Gateway 2000, North Sioux City, SD) with software that allowed monitoring and automated control of brain temperature.

After 2-4 days of recovery, rats were fasted for 12-16 h but allowed free access to water. The animals were anesthetized with isoflurane and surgically prepared as in experiment 1. After surgical preparation, a 20-min interval was allowed for physiologic stabilization. Rectal temperature was monitored using a temperature probe (YSI Telethermometers model 401; Yellow Spring Instrument Co.) during surgical preparation, ischemia, and for 120 min after reperfusion. During surgical preparation, rectal temperature was servo-regulated at 37.5 ± 0.1°C by surface heating and cooling. Thereafter, brain temperature was controlled at 37.5°C by surface heating and cooling during ischemia and for the first 22 h of recovery.

Rats were assigned randomly to the same four groups as described for experiment 1 (n = 15 per group) and then subjected to the identical ischemic injury described for experiment 1. Anesthetic agents were continued after ischemia for variable periods (isoflurane groups = 110 min; fentanyl-N₂O groups = 80 min) to ensure that the animals in all the groups would recover the righting reflex approximately 2 h after reperfusion began. The tracheas were extubated and the rats were placed in an oxygen-enriched recovery chamber (inspiratory fraction = 0.3 to 0.4) to allow temperature control for an additional 22 h. Rats were returned to their cages with free access to water and food for 5 days.

On the fifth postoperative day, with the observer blinded to group assignment, motor function tests were performed according to an established protocol and included assays of phrenic nerve function and balance beam performance. The motor score was graded on a 0-9 scale (best score = 9). Rats were anesthetized with halothane and underwent in situ brain fixation by intracardiac injection of buffered 4% formalin. After 24 h, the brains were removed and stored in 4% formalin. Paraffin-embedded brain sections were cut serially (5 µm thick) and stained with thionin–celestine blue. With the investigator blinded to group assignment, injury to the CA1 sector of the hippocampus was evaluated using the light microscope. Viable and nonviable neurons were counted manually, and the percentage of nonviable neurons was calculated (percentage dead CA1). Where the septal nuclei were widest, damage in the neocortex was graded (crude damage index) on a 0-3 scale (0 = no damaged neurons; 1 = 1-50% neurons damaged; 2 = 50-60% neurons damaged; 3 > 60% of neurons damaged). Values from the hemisphere with the worst damage in each animal were used for the statistical analysis.

In a subset of 40 animals (n = 10 or 11 per group), the heart, liver, and kidneys were removed with the brains 24 h after formalin fixation to detect obvious histologic
Table 1. Physiologic Values (Mean ± SD) for Experiment 1

<table>
<thead>
<tr>
<th></th>
<th>Fentanyl/N₂O</th>
<th>Fentanyl/N₂O + Trimethaphan</th>
<th>Isoflurane</th>
<th>Isoflurane + Trimethaphan</th>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>5</td>
<td>8</td>
<td>5</td>
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<tr>
<td>Preischemia body weight (g)</td>
<td>300 ± 12</td>
<td>293 ± 20</td>
<td>305 ± 10</td>
<td>291 ± 10</td>
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<tr>
<td>MAP (mmHg)</td>
<td>124 ± 13</td>
<td>109 ± 12</td>
<td>94 ± 17</td>
<td>94 ± 8</td>
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<td>pH₅CRT</td>
<td>7.34 ± 0.03</td>
<td>7.35 ± 0.03</td>
<td>7.33 ± 0.04</td>
<td>7.35 ± 0.02</td>
</tr>
<tr>
<td>PₐCO₂ (mmHg)</td>
<td>38 ± 2</td>
<td>38 ± 1</td>
<td>38 ± 1</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>PₐO₂ (mmHg)</td>
<td>144 ± 19</td>
<td>128 ± 12</td>
<td>120 ± 13</td>
<td>129 ± 9</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>144 ± 22</td>
<td>129 ± 20</td>
<td>163 ± 28</td>
<td>158 ± 14</td>
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<tr>
<td>Hematocrit (%)</td>
<td>44 ± 2</td>
<td>44 ± 2</td>
<td>44 ± 2</td>
<td>43 ± 1</td>
</tr>
<tr>
<td>Postischemia (mmHg)</td>
<td>110 ± 18</td>
<td>110 ± 11</td>
<td>94 ± 11</td>
<td>102 ± 12</td>
</tr>
<tr>
<td>pH₅CRT</td>
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<td>7.30 ± 0.08</td>
<td>7.19 ± 0.08</td>
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<td>PₐCO₂ (mmHg)</td>
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<tr>
<td>PₐO₂ (mmHg)</td>
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<td>130 ± 21</td>
<td>121 ± 12</td>
<td>126 ± 13</td>
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<tr>
<td>Glucose (mg/dl)</td>
<td>91 ± 17</td>
<td>115 ± 22</td>
<td>132 ± 23</td>
<td>158 ± 31</td>
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<tr>
<td>Hematocrit (%)</td>
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<td>41 ± 2</td>
<td>42 ± 2</td>
<td>40 ± 1</td>
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<tr>
<td>Postischemia (mmHg)</td>
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<td>95 ± 16</td>
<td>79 ± 10</td>
<td>84 ± 13</td>
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<td>pH₅CRT</td>
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<td>PₐCO₂ (mmHg)</td>
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<td>40 ± 2</td>
<td>38 ± 3</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>PₐO₂ (mmHg)</td>
<td>143 ± 25</td>
<td>126 ± 20</td>
<td>119 ± 19</td>
<td>137 ± 17</td>
</tr>
</tbody>
</table>

signs of ischemic damage. Paraffin-embedded organ sections were cut serially (5 μm thick) and stained with hematoxylin and eosin. A pathologist blinded to group assignment graded signs of ischemic damage in these organs (0 = no definite evidence of ischemic damage; 1 = patchy to moderate damage; 2 = widespread damage).

Statistical Analysis

For experiment 1, the statistically analyzed end point was a change in plasma catecholamine concentrations from 2 min before to 2 min after ischemia onset. For experiment 2, cortical and hippocampal CA1 damage and total motor scores were compared among groups. Statistical comparisons were chosen a priori and were performed using the Wilcoxon-Mann-Whitney U test. The two-sided P values were exact (StatXact 3 for Windows; Cytel Software, Cambridge, MA) and were corrected for the effect of multiple comparisons using the Bonferroni method. We used a distribution-free method for two reasons. First, the catecholamine data from experiment 1 had 1) unequal sample sizes among groups, 2) markedly different variances among groups, and 3) some zero values (which prevented the use of logarithmic transformation of the change in concentrations). Second, cortical histologic damage and motor scores were measured using ranked scales. We tested for effects of random variation of physiologic variables on histologic outcome by using analysis of covariance. Correlation between hippocampal CA1 and renal injury was assessed using Kendall's τ; with two-sided P values. Statistical significance was assumed when P < 0.05.

Results

Experiment 1

Table 1 shows physiologic values. Mean arterial pressure tended to be less in rats anesthetized with isoflurane, whereas glucose values tended to be greater. Post-ischemic pH values tended to be greater in those animals given trimethaphan. Post hoc analysis of covariance did not detect a significant effect of any measured physiologic variable on changes in norepinephrine or epinephrine plasma concentrations. During the ischemic interval, electroencephalographic isoelectricity was present in all animals that completed the experimental protocol. Pericranial temperature was controlled at 37.5 ± 0.2°C as intended. Figure 1 shows the time course for plasma norepinephrine and epinephrine concentrations. Before and after ischemia, norepinephrine and epinephrine concentrations were similar among groups. Fifty-fold increases in norepinephrine and 132-fold increases in epinephrine were observed in the fentanyl-N₂O group during ischemia. Isoflurane anesthesia caused a substantial reduction in the magnitude of the release of norepinephrine and epinephrine when compared with fentanyl-
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Fig. 1. The time course for plasma norepinephrine (A) and epinephrine (B) concentrations before, during, and after near-complete global cerebral ischemia (mean ± SD). Filled circles = fentanyl-nitrous oxide (N₂O); open circles = fentanyl-N₂O + trimethaphan; filled squares = isoflurane; open squares = isoflurane + trimethaphan. Isoflurane anesthesia caused a substantial reduction in the magnitude of the adrenergic response when compared with fentanyl-N₂O (corrected $P = 0.002$ for norepinephrine and corrected $P = 0.01$ for epinephrine). Trimethaphan abolished any changes in circulating catecholamines caused by the onset of ischemia in both the fentanyl-N₂O- and isoflurane-anesthetized rats.

$N₂O$ (corrected $P = 0.002$ for norepinephrine; corrected $P = 0.01$ for epinephrine). Values for isoflurane anesthesia alone were 2 and 11 times greater for norepinephrine and epinephrine, respectively, compared with those observed in rats given isoflurane + trimethaphan (corrected $P = 0.02$ for norepinephrine and epinephrine). Trimethaphan blocked ischemia-related changes in plasma catecholamine concentrations for both anesthetics evaluated (corrected $P \leq 0.02$ for each of the four comparisons).

Experiment 2

Two animals died of undefined causes on the third day after ischemia in the fentanyl-N₂O group. One rat in the isoflurane + trimethaphan group developed seizures and was killed on the second day after ischemia. These three animals were replaced to keep sample sizes equal. Table 2 shows the physiologic values for animals in experiment 2. Again, mean arterial pressure tended to be less in rats anesthetized with isoflurane, whereas glucose values tended to be greater. Postischemic pH values tended to be greater in those animals given trimethaphan. During the ischemic interval, electroencephalographic isoelectricity was present in all animals included in later analyses. Rectal and brain temperatures were controlled as intended. At all times, in each of the four groups, the cortical temperature among the animals never varied significantly, at $37.5 ± 0.2^\circ C$ throughout.

Figure 2 shows hippocampal CA1 injury. Trimethaphan did not affect the percentage (mean ± SD) of dead CA1 neurons in rats anesthetized with fentanyl-N₂O (fentanyl-N₂O = 87 ± 10%; fentanyl-N₂O + trimethaphan = 87 ± 6%; corrected $P = 1.00$). Isoflurane substantially reduced the percentage of dead CA1 neurons compared with fentanyl-N₂O (isoflurane = 43 ± 22%; fentanyl-N₂O = 87 ± 10%; corrected $P < 0.001$). The relative protective effect of isoflurane was abolished by trimethaphan (isoflurane = 43 ± 22%, isoflurane + trimethaphan = 91 ± 6%; corrected $P < 0.001$). We did not detect a significant difference between fentanyl-N₂O and isoflurane in the presence of trimethaphan ($P = 0.40$). Analysis of covariance did not detect a significant effect of any measured physiologic variable on CA1 damage.

For the cortex, we detected a difference in the crude damage index (median ± interquartile deviation) between isoflurane and fentanyl-N₂O-anesthetized animals (fentanyl-N₂O = 2 ± 0; isoflurane = 1 ± 0; corrected $P < 0.001$; fig. 3). Again, the protective effect of isoflurane was prevented by trimethaphan (isoflurane = 1 ± 0; isoflurane + trimethaphan = 2 ± 0; corrected $P < 0.001$). Post hoc analysis of covariance detected an effect of the oxygen tension in arterial blood ($PaO_2$) 10 min before ischemia on the crude damage index for the motor cortex (uncorrected $P = 0.007$). The effect of $PaO_2$ 10 min before ischemia was to increase the significant $P$ values (i.e., make the values “less significant”). We did not detect a significant difference among...
groups for the motor scores, which are shown in figure 4. Post hoc analysis of covariance did not detect a significant effect of any measured physiologic variable on the total motor score.

The hearts and livers showed virtually no histologic signs of ischemic damage. However, in a few livers in rats evenly distributed among groups, we did find some nonspecific granulomas that did not have a centriflobular pattern. Animals receiving fentanyl showed signs of resolving acute tubular necrosis in the kidneys, dissimilar to animals that received isoflurane (median ± quartile deviation fentanyl 2 ± 0, fentanyl plus trimethaphan 1 ±

![Fig. 2. Circles depict the percentage of dead hippocampal CA1 neurons in rats as determined 5 days after near-complete forebrain ischemia. Horizontal bars depict group mean values. Isoflurane caused reduced damage after near-complete ischemia when compared with fentanyl-N2O (corrected P < 0.001). Trimethaphan (TMP) abolished the relative protective effect of isoflurane (corrected P < 0.001). In fentanyl-N2O-anesthetized rats, trimethaphan administration did not change the histologic result.](image)

![Fig. 3. The severity of histologic damage in the neocortex after near-complete forebrain ischemia. Circles depict a crude damage index for individual rats. Horizontal bars depict median values. Isoflurane caused reduced damage after near-complete ischemia when compared with fentanyl-N2O. Again, trimethaphan (TMP) worsened the histologic result in isoflurane-treated animals. There was no effect of trimethaphan on histologic outcome in fentanyl-N2O-anesthetized rats. 0 = no damaged neurons; 1 = 1–30% neurons damaged; 2 = 30–60% neurons damaged; and 3 = more than 60% of neurons damaged.](image)
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![Diagram showing total motor score for different treatments.](image)

Fig. 4. The total motor score determined 5 days after near-complete forebrain ischemia. Each open circle depicts values for a single rat. Horizontal bars denote the median value for each group. 9 = no deficits. Motor function tests did not detect a difference among groups. TMP = trimethaphan.

0, isoflurane 0 ± 0, isoflurane plus trimethaphan 0 ± 0. Overall, there was no correlation between renal and hippocampal injury (Kendall’s τ = 0.11; P = 0.38; 95% CI = 0.13 to 0.35). However, we did not detect a significant correlation between renal and hippocampal injury among rats receiving fentanyl (Kendall’s τ = 0.46; P = 0.10), fentanyl plus trimethaphan (Kendall’s τ = 0.49; P = 0.11), or fentanyl with or without trimethaphan (Kendall’s τ = 0.29; P = 0.11).

Discussion

The results of this study confirm that histologic outcome from severe global cerebral ischemia is better in rats anesthetized with isoflurane compared with those anesthetized with fentanyl-N₂O. At the same time, anesthetic agents differentially modulated the peripheral adrenergic response to ischemia. This adrenergic response was prevented effectively by the sympathetic blocking agent trimethaphan. Sympathetic ganglionic blockade also reversed the relative neuroprotective effects of isoflurane on outcome but did not change the histologic outcome in rats anesthetized with fentanyl-N₂O.

Known physiologic determinants of outcome from global ischemia were measured in experiments 1 and 2. Of these, a consistent pattern of lower preischemic and posts ischemic mean arterial pressure was observed in the isoflurane groups. However, this could not be associated with a significant effect on outcome as a covariate. Furthermore, there is evidence that substantially greater differences in mean arterial pressure are necessary to alter histologic outcome in this model.22 Finally, mean arterial pressure was held similarly at 30 mmHg during ischemia in all groups. Analysis of covariance detected an effect of preischemic PaO₂ on cortical but not hippocampal injury (i.e., the higher the preischemic PaO₂, the lower the cortical crude damage index score). Therefore, if anything, this caused an underestimation of the significance of the difference in cortical injury between isoflurane and the three other groups, because PaO₂ values for the isoflurane group were the lowest numerically.

Differences among groups for plasma glucose before ischemia were relatively modest and did not reach the threshold required to affect outcome from cerebral ischemia, as previously described for this rodent model.23 Numerically lower glucose levels during reperfusion in the fentanyl-N₂O-treated animals that were not given trimethaphan may have resulted from partial depletion of glycogen stores caused by the more pronounced catecholamine stimulation seen in this group.24,25

Our first experiment was performed to determine whether concentrations of circulating catecholamines during severe global ischemia depend on the anesthetic administered and whether 2.5 mg trimethaphan given intravenously would be adequate to suppress any increase in circulating catecholamine levels. The dramatic increase in plasma norepinephrine and epinephrine at 2 min of ischemia in the fentanyl-N₂O-anesthetized rats clearly was attenuated by isoflurane. This is consistent with the findings of Hoffman et al.4,18 who also found that halothane, isoflurane, and ketamine anesthesia suppressed plasma catecholamine concentrations (compared with fentanyl-N₂O) when measured at the end of a 30-min episode of hemispheric ischemia. That trimethaphan can provide potent inhibition of the release of catecholamines into the circulation is also consistent with the work of others. Smith et al.18 reported large increases of peripheral catecholamine levels obtained in a model of less severe forebrain ischemia in rats slightly sedated with N₂O, and they found that trimethaphan markedly attenuated those changes.

Our second experiment was designed to determine whether sympathetic ganglionic blockade could alter histologic outcome from nearly complete ischemia by preventing the peripheral adrenergic response to ischemia during isoflurane or fentanyl-N₂O anesthesia. This was true for rats anesthetized with isoflurane. Rats administered isoflurane plus trimethaphan had substantially worsened histologic outcome compared with rats given isoflurane alone. In contrast to the work of Werner
et al., 9 who showed that ganglionic blockade improved outcome in rats sedated with N2O, we could not detect a difference between fentanyl-N2O-anesthetized rats given trimethaphan and rats in which the spontaneous adrenergic response was allowed. Clearly, trimethaphan did not improve outcome. However, because hippocampal damage was so severe, we could not conclude that trimethaphan would not worsen outcome in fentanyl-N2O-treated rats if the ischemic insult was of shorter duration.

Our histologic findings are consistent with recent work by Miura et al., 6 who showed better histologic outcome in isoflurane-anesthetized rats compared with rats anesthetized with ketamine or fentanyl-N2O. At the same time, the current experiment explains the discrepancy between the recent study by Miura et al. and earlier work by Warner et al. 20 In that study, isoflurane offered no advantage over N2O alone for histologic outcome from 10 min of severe forebrain ischemia. Warner et al. 20 administered trimethaphan, which we propose may have masked any neuroprotective effect that isoflurane might have offered.

Our histologic findings did not parallel the results of the motor function test. This warrants consideration. Given the absence of cortical infarcts in the four experimental groups, the relatively high scores in the basic neuromotor function test described by Combs and D'Alsey 19 are not surprising. 19 Previous work has shown that this test is not sufficiently sensitive to detect behavioral differences in the presence of selective neuronal necrosis only. 23 More sensitive assays, including performance in a radial maze, are needed to detect these differences.

We are left to ponder why blocking the adrenergic response masks the relative protective effect of isoflurane. In a study in mongrel dogs, Magness et al. 27 reported significant trimethaphan-related electroencephalographic changes that were unrelated to hypotension and suggested a possible cerebral toxic effect of trimethaphan. Trimethaphan does not appear to enter the central nervous system but has been shown to decrease the concentration of norepinephrine in the cerebrospinal fluid. 28,29 It is possible that systemically administered trimethaphan and circulating catecholamines can reach and influence circumventricular regions, such as the area postrema or subfornical organ, which lack an effective blood–brain barrier and may contribute in part to the regulation of sympathetic outflow within the brain. 30 This would be consistent with the findings of Blomqvist et al., 11 who showed that inflicting lesions on the locus ceruleus worsens outcome from forebrain ischemia. To our knowledge, experiments have not been performed in vitro that assayed any neuroprotective properties of norepinephrine or epinephrine on simulated ischemic injury at the neuronal level.

It is unlikely that the neuroprotective effects of isoflurane in this study resulted from cerebral blood flow differences among the study groups. Cerebral blood flow studies during nearly complete forebrain ischemia consistently have found flow reduced to less than 5% of control values. 17,18 Furthermore, in a recent study in rats, Miura et al. 6 showed that the near-complete ischemic insult, as used in the current study, is uniformly severe among subjects in different anesthetic groups. Cortical and hippocampal electroencephalographic recordings were homogeneous with respect to the presence or absence of isoelectricity, and all animals underwent ischemic depolarization. However, differential effects of anesthetic agents on delayed posts ischemic hypoperfusion are largely unexamined. This may warrant further study.

We could argue that the modulating effect of isoflurane on the sympathetic response to ischemia represents just one of many actions that in concert explain the repeated demonstration of its neuroprotective properties. 3,4,6 Volatile anesthetics directly protect neurons from excitotoxic injury and seem to reduce spreading and depression-like depolarizations in ischemic brain tissue. 51,52 Isoflurane reduces the ion flux stimulated by N-methyl-D-aspartate in cortical cell culture and decreases the calcium influx in synaptosomes stimulated by N-methyl-D-aspartate. 33,34 Recently, isoflurane was shown to reduce the frequency of transient ischemic depolarizations, which are thought to contribute to ischemic damage. 35 Finally, isoflurane reduces the cerebral metabolic rate, thereby preserving high-energy phosphate concentrations. 46

In conclusion, in rats subjected to an ischemic insult severe enough to cause electroencephalographic isoelectricity, isoflurane partially inhibited the peripheral sympathetic response to ischemia and improved histologic outcome. Trimethaphan completely prevented any ischemia-induced increases in circulating catecholamines and reversed the protective effect of isoflurane. Relevant physiologic values were controlled adequately. The beneficial effects of isoflurane may be the result of a positive influence from an intermediate sympathetic response that is abolished by trimethaphan.

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