

A Comparison of the Cerebral Protective Effects of Isoflurane and Mild Hypothermia in a Model of Incomplete Forebrain Ischemia in the Rat

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The present study was undertaken to examine the cerebral protective properties attributed to isoflurane and at the same time to compare its protective effects with those of mild hypothermia (temperature reduction by 3° C). Twenty-one fasted Wistar-Kyoto rats were assigned to one of three groups (n = 7): 1.3 MAC (end-tidal) isoflurane–normothermia (pericranial temperature 38.0° C), 1.3 MAC halothane–normothermia, and 1.3 MAC halothane–hypothermia (pericranial temperature 35.0° C during ischemia). In each animal the trachea was intubated and the lungs were mechanically ventilated. Each animal was subjected to temporary incomplete forebrain ischemia induced by 10 min of bilateral carotid artery occlusion with simultaneous hypotension (mean arterial pressure 35 mmHg) induced by trimetaphan and blood withdrawal. After a 3-day survival period, perfusion–fixation was performed, and two blinded observers assessed histopathologic injury according to a four-point scale (0 = no damage; 1 = <10% of neurons damaged; 2 = 10–50% damaged; and 3 = >50% damaged). The assessment was performed at two points in the rostrocaudal axis chosen to permit

evaluation of regions with varying levels of ischemic damage. In the rostral sections, in the isoflurane- and halothane–normothermia groups, moderate to severe injury was observed in striatum, cerebral cortex, hippocampus (CA1 and CA3 areas), and reticular nucleus of the thalamus (e.g., the median scores for the CA1 area were 3 in both the halothane–normothermia and the isoflurane–normothermia groups), and there were no differences between the two groups. By contrast, the halothane–hypothermia group showed significantly less damage (e.g., the median score for the CA1 area was 1) in all but the hippocampal CA3 area. In the caudal section, the injury was significantly less severe in portions of the cortex and hippocampal CA1. As in the rostral sections, the degree of damage did not differ between the two normothermia groups, but was significantly less severe in the halothane–hypothermia group. The data indicate that in the circumstances of the present study, isoflurane did not confer protection relative to control animals anesthetized with an equipotent concentration of halothane, but that mild intraintracerebral hypothermia (by 3° C) was markedly protective. (Key words: Anesthetics, volatile: halothane; isoflurane. Anesthetic techniques: hypothermia. Brain: ischemia.)

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ISOFLURANE CAN PRODUCE a depression of cerebral metabolic rate (CMR) equivalent to that attainable with barbiturates.^{1,2} Accordingly, it is reasonable to suspect that isoflurane might also have cerebral protective properties. It has been shown that isoflurane slows the rate of depletion of cerebral energy metabolites during incomplete global ischemia³ and that critical cerebral blood flow (CBF) during carotid endarterectomy is lower during anesthesia with isoflurane than with other anesthetic agents.⁴ However, attempts to demonstrate, using histologic endpoints, the protective effects of isoflurane have been largely,^{5–10} although not exclusively,^{11,12} unsuccessful. Accordingly, there is ongoing interest in achieving a convincing laboratory demonstration of the cerebral protective effects of isoflurane.

There have also been several demonstrations that mild hypothermia (2–3° C) has a substantial cerebral protective effect against ischemic injury.^{13–15} The use of mild hypothermia as a cerebral protective technique during anesthesia has potential advantages. Provided that re-warming is accomplished by the time of emergence, the cardiovascular effects are minimal. In addition, it is not associated with a prolonged postoperative influence on

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the level of consciousness, as may occur after barbiturate administration. The present study was undertaken to examine further the question of isoflurane's efficacy as a cerebral protectant and, in addition, to provide a comparison of isoflurane's protective effect with that of a mild (3° C) reduction in body temperature.

Materials and Methods

The study was approved by the institutional animal use committee. Thirty-one male Wistar-Kyoto rats were obtained concurrently from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The animals were of the same age and weighed 275–350 g.

MAC DETERMINATION

Eight Wistar-Kyoto rats were randomly assigned to receive either isoflurane ($n = 4$) or halothane ($n = 4$). MAC was determined as described by Cole *et al.*¹⁶ MAC values (means \pm SD) were $1.15 \pm 0.05\%$ for isoflurane and $0.78 \pm 0.04\%$ for halothane.

ISCHEMIA STUDIES

After an overnight fast with unrestricted access to water, the animals were allocated randomly to one of three groups: isoflurane–normothermia, halothane–normothermia, or halothane–hypothermia. Animals in the isoflurane–normothermia group were anesthetized with 4% isoflurane in oxygen and those in the halothane–normothermia and halothane–hypothermia groups with 3% halothane in oxygen. After tracheal intubation, the lungs were mechanically ventilated at a respiratory rate of 60 breaths \cdot min⁻¹ with a tidal volume of 12 ml \cdot kg⁻¹. Anesthesia was maintained with 2% isoflurane (isoflurane–normothermia group) or 1.3% halothane (halothane–normothermia and halothane–hypothermia groups) in 40% oxygen/balance nitrogen. Carbon dioxide was added to the inspired gas mixture, and its concentration was adjusted as necessary to maintain normocapnia (P_{aCO_2} 35–40 mmHg).

End-tidal anesthetic and CO₂ concentrations were monitored by an infrared gas analyzer (Ohmeda 5250 RGM). A thermistor probe (YSI Reusable Temperature Probe, Yellow Springs Instrument Co., Inc., Yellow Springs, OH) was placed in the distal esophagus, and esophageal temperature was controlled by servomechanism (YSI model 73ATA, Yellow Springs Instrument Co., Inc.) at 38° C during the surgical preparation. A thermocouple probe (Hi-Lo Temp[®], Mallinckrodt Inc., Glens Falls, NY) inserted beneath the scalp was used to monitor pericranial temperature. A PE-50 catheter was inserted into the tail artery for continuous monitoring of arterial pressure and for withdrawal of samples of arterial blood.

A midline pretracheal skin incision was made. A PE-60 catheter was placed in the superior vena cava *via* the right external jugular vein for blood volume manipulation and drug administration. Both carotid arteries were exposed and separated carefully from the cervical sympathetic and vagus nerves. A loose silk suture was placed around each artery. All wounds were infiltrated with 0.25% bupivacaine (Sensorcaine[®], Astra Pharmaceutical Products, Westborough, MA).

After surgical preparation, the animal was placed in the supine position with the head and neck within a clear Plexiglas chamber specifically designed for maintenance of pericranial temperature.¹⁷ The chamber was 15 \times 9 \times 9 cm. The animal entered the chamber *via* a latex diaphragm. The chamber was flushed continuously with warm humidified air (MR450 SERVO[®], Fisher & Pykel Ltd., Auckland, New Zealand), controlled by servomechanism *via* a thermistor within the box, to the pericranial target temperature (35 or 38° C). This technique, which maintains high humidity and a temperature much closer to brain temperature than to that of normal ambient air (approximately 21° C), has been shown to minimize brain temperature fluctuation during episodic ischemia in rats.¹⁷ The chamber has a removable top that can be opened to facilitate cooling or to permit application of ice packs. The end-tidal concentration of volatile anesthetic was then reduced to 1.3 MAC (1.5% isoflurane/1.0% halothane).

In the two normothermia groups, esophageal temperature was controlled by servomechanism at 38° C using a heat lamp directed at the body. Pericranial temperature, which without additional measures was invariably less than the esophageal temperature, was also maintained at $38.0 \pm 0.2^\circ$ C (Hi-Lo Temp[®] model 8200) using a second heat lamp directed at the Plexiglas chamber. In the halothane–hypothermia group, both servomechanisms were reset to 35° C, and malleable ice packs (6 \times 6 \times 1 cm) were applied to the lateral aspects of the shoulders, neck, and skull. When pericranial temperature reached 35.0° C, the ice packs were removed. The pericranial temperature then was maintained at $35.0 \pm 0.2^\circ$ C. This was accomplished by activation of the heat lamp directed at the Plexiglas chamber when the temperature was less than 35.0° C and by reapplication of the ice packs when it was greater than 35.0° C, although the latter was rarely necessary. The protocol provided for application of an ice pack to the abdomen in the event of an esophageal temperature greater than 35.5° C, but this maneuver was never required. After the target pericranial temperature had been achieved, a 10-min equilibration period ensued. Mean arterial pressure (MAP) and heart rate (HR) were recorded, and arterial blood was withdrawn for determination of PaO₂, PaCO₂, pH, hematocrit, and plasma glucose. These are referred to as the preischemia values.

Forebrain ischemia was induced according the technique of Smith *et al.*¹⁸ Thirty units of heparin were ad-

ministered intravenously. Five minutes later, hypotension was induced by intravenous administration of 2–4 mg trimetaphan (Arfonad[®], Roche Laboratory, Nutley, NJ) followed by withdrawal of blood through the external jugular vein catheter into a prewarmed syringe. When MAP had been reduced to 35 mmHg, both carotid arteries were occluded with vascular clamps. The occlusion was maintained for a period of 10 min, during which MAP was maintained at 35 mmHg by further exsanguination or by reinfusion of the withdrawn blood. At the end of the 10-min period of ischemia, reperfusion of the brain was established by removal of vascular clamps with simultaneous normalization of the MAP by rapid reinfusion of the withdrawn blood. Protamine sulfate (QUAD Pharmaceuticals, Indianapolis, IN) 0.3 mg was administered intravenously to reverse the heparin effect. Sodium bicarbonate (0.45 mEq/ml) 0.25 mEq was administered *via* the arterial line to counteract the metabolic acidosis associated with the period of hypotension. The ischemic insult parameters (10 min of bilateral carotid artery occlusion at MAP 35 mmHg) were developed in preliminary investigations of normothermic 1.3 MAC isoflurane-anesthetized Wistar-Kyoto rats. The parameters were chosen to be just below the threshold for the occurrence of gross neurologic deficits other than the high-arched gate (probably a reflection of minor spasticity) previously described by Smith *et al.*¹⁸

Rewarming of the animals in the halothane–hypothermia group to esophageal and pericranial temperatures of 38° C began immediately upon reperfusion and was accomplished within 5 min by reinfusion of warmed withdrawn blood and application of a heat lamp. MAP and HR were recorded 5 min after reperfusion; these are referred to as the postischemia values. The vascular catheters and the pericranial temperature probe were removed, and the neck and tail incisions were sutured. Anesthetic administration was discontinued, and the lungs were ventilated with 100% oxygen. Esophageal temperature was controlled by servomechanism at 38° C in all animals until discontinuation of the anesthetic, at which time the esophageal probe was removed and a rectal probe was inserted. Upon resumption of spontaneous ventilation, the animals were transferred to a prewarmed incubator that was flushed continuously with humidified oxygen. Extubation of the trachea was performed when spontaneous movement occurred. Rectal temperature was controlled by servomechanism at 38° C for approximately 1 h after discontinuation of the anesthetic. The animals were observed continuously for 3 h, by which time all had recovered mobility.

HISTOPATHOLOGIC EXAMINATION

Three days after the ischemic insult, the animals were reanesthetized with 4% isoflurane in 100% oxygen. Transcardiac perfusion–fixation was performed with 50

ml heparinized saline followed by 50 ml 10% phosphate-buffered formalin. The brains were allowed to remain *in situ* for 30 min and then were removed and refrigerated in 10% phosphate-buffered formalin for 24–48 h. After dehydration in graded concentrations of ethanol and butanol, the brains were embedded in paraffin. Coronal sections of the brain were cut at a thickness of 8 μm and stained with hematoxylin and eosin. Ten regions of brain (fig. 1) were examined semiquantitatively for histologic evidence of injury. These included regions that were centrally located within the ischemic territory as well as more posterior regions near its periphery, and included both cortical and subcortical structures. Dorsolateral striatum was evaluated in a coronal plane 600 μm posterior to the bregma (fig. 1, section a). Parietal cortex, the reticular nucleus of the thalamus, and the CA1 and CA3 sectors of the hippocampus were evaluated in a coronal plane 3300 μm posterior to the bregma (fig. 1, section b). Temporal-occipital cortex, entorhinal cortex, the CA1 sector of both the dorsal and the ventral hippocampus, and the CA3 sector of hippocampus were evaluated in a plane

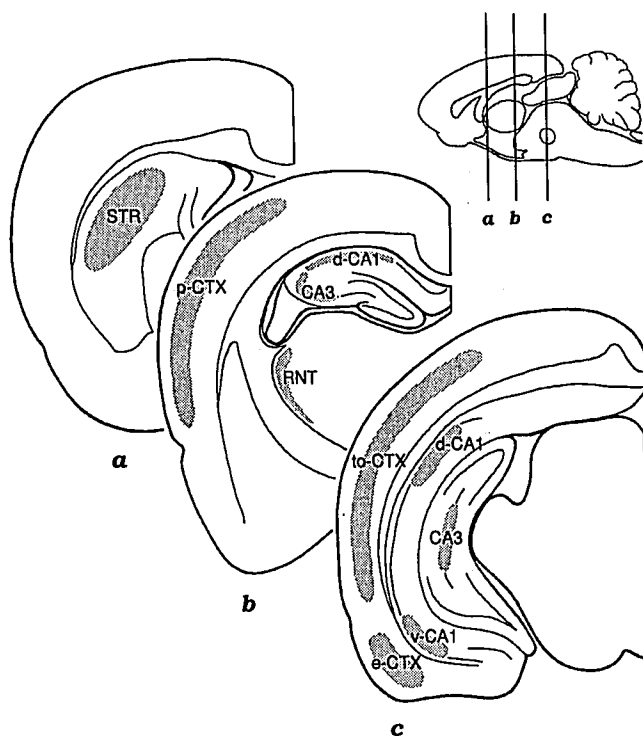


FIG. 1. Representations of the coronal sections of rat brain evaluated for histopathologic injury 3 days after forebrain ischemia. The rostro-caudal locations of the three coronal sections (a, b, and c) are shown in the sagittal cross-sectional image (upper right). The shaded areas represent the regions of each coronal section in which histologic grading was performed. STR = dorsolateral striatum; p-CTX = parietal cortex; d-CA1 = CA1 sector of the dorsal hippocampus; v-CA1 = CA1 sector of the ventral hippocampus; CA3 = CA3 sector of the hippocampus; RNT = reticular nucleus of thalamus; to-CTX = temporal-occipital cortex; e-CTX = entorhinal cortex.

6000 μm posterior to the bregma (fig. 1, section c). The three coronal planes correspond approximately to plates 21, 30, and 39 in the atlas of Palkovits and Brownstein.¹⁹

Neuronal injury was evaluated at a magnification of 400 \times by two observers who had no knowledge of the treatment groups. Ischemic neurons were identified by cytoplasmic eosinophilia with loss of Nissl substance and by the presence of pyknotic homogeneous nuclei. In each region the frequency of ischemic neurons was graded on a scale of 0 to 3, where 0 = no ischemic neurons; 1 = less than 10% ischemic neurons; 2 = 10–50% ischemic neurons; and 3 = greater than 50% ischemic neurons.

STATISTICAL ANALYSIS

The physiologic variables were analyzed by a two-way analysis of variance. Where differences were identified, *post hoc t* tests (paired *t* tests for intragroup comparisons and unpaired *t* tests for intergroup comparisons) with Bonferroni corrections for multiple comparisons were performed. The histologic scores were analyzed using a nonparametric two-factor analysis of variance.²⁰ Where significant differences were demonstrated, *post hoc* tests were performed as follows. Intergroup comparisons for each region were performed using the Mann-Whitney U test with Bonferroni corrections for multiple comparisons, and intragroup comparisons (performed to confirm the decreasing severity of the ischemic insult along the rostrocaudal axis) were performed using the Wilcoxon signed rank test. A *P* value of less than 0.05 was considered statistically significant.

Results

The physiologic data are presented in table 1. The weights of the animals did not differ among the groups. Preischemic MAP and HR did not differ between the two normothermic groups prior to ischemia. However, MAP and HR were significantly less in the halothane-hypothermia group than in the isoflurane-normothermia group. At the postischemic measurement interval, MAP was significantly less in the halothane-normothermia and halothane-hypothermia groups than in isoflurane-normothermia animals. Preischemic PaO_2 was greater in the halothane-hypothermia group than in the halothane-normothermia group but did not differ between the halothane-hypothermia and isoflurane-normothermia groups. There were no differences in PaCO_2 , *pH*, hematocrit, or plasma glucose.

Two animals died in the early postischemic period, one in the halothane-normothermia group with apparent upper airway obstruction and one in the isoflurane-normothermia group following a seizure. These animals were replaced with two additional animals in order to maintain balanced groups ($n = 7$ per group). All of the 21 surviving

TABLE 1. Mean Arterial Pressure and Heart Rate Preischemia and 5 min Postischemia; Arterial Blood Gases, Hematocrit and Plasma Glucose Preischemia

	Isoflurane 38° C	Halothane 38° C	Halothane 35° C
Number of animals	7	7	7
Weight (g)	319 \pm 15	318 \pm 34	331 \pm 34
MAP preischemia (mmHg)	103 \pm 14	94 \pm 14	85 \pm 7*
MAP postischemia (mmHg)	101 \pm 19	67 \pm 13†‡	77 \pm 12†*
HR preischemia (beats/min)	389 \pm 43	351 \pm 20	327 \pm 48*
HR postischemia (beats/min)	357 \pm 45	354 \pm 10	331 \pm 23
PaO_2 (mmHg)	198 \pm 25	143 \pm 25	217 \pm 57§
PaCO_2 (mmHg)	36 \pm 1.5	37 \pm 2.6	36 \pm 3.0
<i>pH</i>	7.44 \pm 0.02	7.42 \pm 0.03	7.45 \pm 0.05
Hematocrit (%)	45 \pm 2	43 \pm 3	42 \pm 2
Plasma glucose (mm)	9.0 \pm 1.8	7.1 \pm 1.6	7.5 \pm 1.5

All data are presented as mean \pm SD.

* *P* < 0.05, isoflurane 38° C versus halothane 35° C.

† *P* < 0.05, preischemia versus postischemia.

‡ *P* < 0.05, isoflurane 38° C versus halothane 38° C.

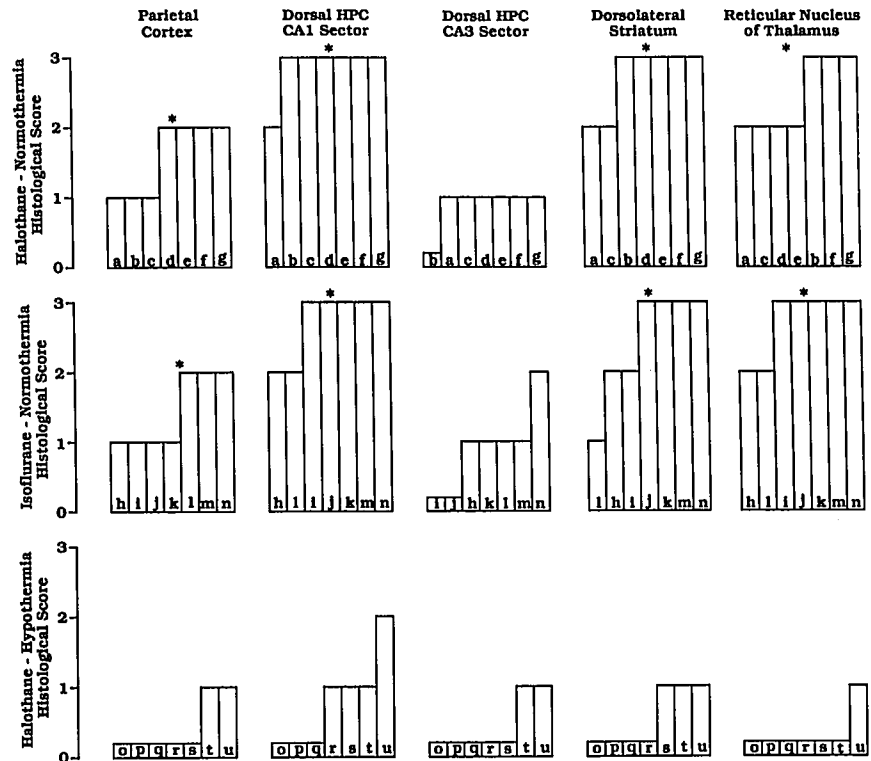
§ *P* < 0.05, halothane 38° C versus halothane 35° C.

animals resumed ambulation within the initial 4-h postischemia observation period, although most appeared docile. By the morning of the day after the ischemic episode, all animals had resumed intake of food and water, and all were rearing and manifested apparently normal levels of activity within their cages. All animals had normal gross neurologic function on the day of perfusion-fixation.

The histopathologic scores for the rostral and caudal sections are presented in figures 2 and 3, respectively. In the rostral sections (fig. 1, sections a and b), there were no differences in histologic scores between the halothane-normothermia and isoflurane-normothermia groups for any of the five regions examined (parietal cortex, dorsolateral striatum, reticular nucleus of thalamus, and CA1 and CA3 sectors of the hippocampus). By contrast, there was markedly less histologic damage in the halothane-hypothermia group in the parietal cortex (*P* < 0.003), the CA1 sector of the hippocampus (*P* < 0.0006), the dorsolateral striatum (*P* < 0.0009), and the reticular nucleus of the thalamus (*P* < 0.0005) than in the two normothermia groups. In fact, three animals in the halothane-hypothermia group had no evidence of injury in any of the five areas examined. Only the CA3 region did not differ significantly among the groups, but it was in this region that the least histologic injury was evident in the normothermic groups.

The histologic scores for the structures in the most caudal section (fig. 1, section c) are presented in figure 3. This section provided the gradation in the severity of histologic injury that was intended by protocol design. For instance, in both normothermic groups, the damage in

FIG. 2. Histologic grade in the rostral sections (sections a and b in fig. 1) in each of five structures in the three experimental groups: halothane-normothermia (top); isoflurane-normothermia (middle); and halothane-hypothermia (bottom). Each bar represents data for one animal, and each animal is identified by a letter of the alphabet. The grading scale was: 0 = no ischemic neurons; 1 = less than 10% ischemic neurons; 2 = 10–50% ischemic neurons; 3 = greater than 50% ischemic neurons. *A significant difference ($P < 0.05$) for that structure between the halothane- or isoflurane-normothermia group and the halothane-hypothermia group.



the entorhinal cortex was significantly less than that observed in the temporal-occipital cortex. In addition, in both normothermia groups, damage to CA1 area of the ventral hippocampus was less severe than that observed in the CA1 area of the dorsal hippocampus. In section c, as was the case with sections a and b, there were no differences in the histologic scores between the halothane-normothermia and isoflurane-normothermia groups. However, once again, there was significantly less damage in the halothane-hypothermia group. The differences were significant in all of the five regions examined. Very little damage was evident in the halothane-hypothermia group. The neuronal injury was limited to grade-1 injuries in the temporal-occipital cortex (two animals) and in the CA1 sector of the dorsal hippocampus (two animals). In four animals, no ischemic damage was evident in any of the five regions.

Discussion

The present study confirms the substantial protective effects of modest hypothermia reported by others¹³⁻¹⁵ but does not provide support for the cerebral protective properties attributed to isoflurane. Among the numerous previous investigations of the protective effects of isoflurane, the most provocative is that of Michenfelder *et al.*²¹ That study, which reviewed more than 2,000 carotid endarterectomy procedures, revealed that the "critical"

CBF, the CBF at which EEG signs of ischemia were likely to appear, was less during anesthesia with isoflurane than during anesthesia with either enflurane or halothane, and thereby suggested that isoflurane was increasing the brain's tolerance for ischemia. That study, however, as acknowledged by the authors, was limited in that it was retrospective and the anesthetic groups nonconcurrent. Furthermore, that study did not reveal differences in neurologic outcome among the various anesthetic groups. As a result, there has been considerable interest in laboratory studies to corroborate, using histopathologic endpoints, the protective effect of isoflurane.⁵⁻¹⁰ Of those investigations have yielded negative results.⁵⁻¹⁰

Warner *et al.*, using a forebrain ischemic insult similar to that used in the present study, demonstrated that isoflurane in concentrations sufficient to produce EEG burst-suppression (3–4% inspired) had no effect on delayed neuronal death relative to that observed in a nitrous oxide sedated control state.⁵ Nehls *et al.*, using a model of middle cerebral artery occlusion (MCAO) in baboons, compared the protective effects of thiopental and isoflurane relative to a nitrous oxide/narcotic-anesthetized control state.⁶ They reported a reduction in infarct volume with thiopental and no difference with isoflurane. However, that study also was criticized because of differences between groups in blood pressure and pressor doses. Gelb *et al.* performed induced hypotension in monkeys during a 45-min period of MCAO.⁷ Induction of hypotension was ac-

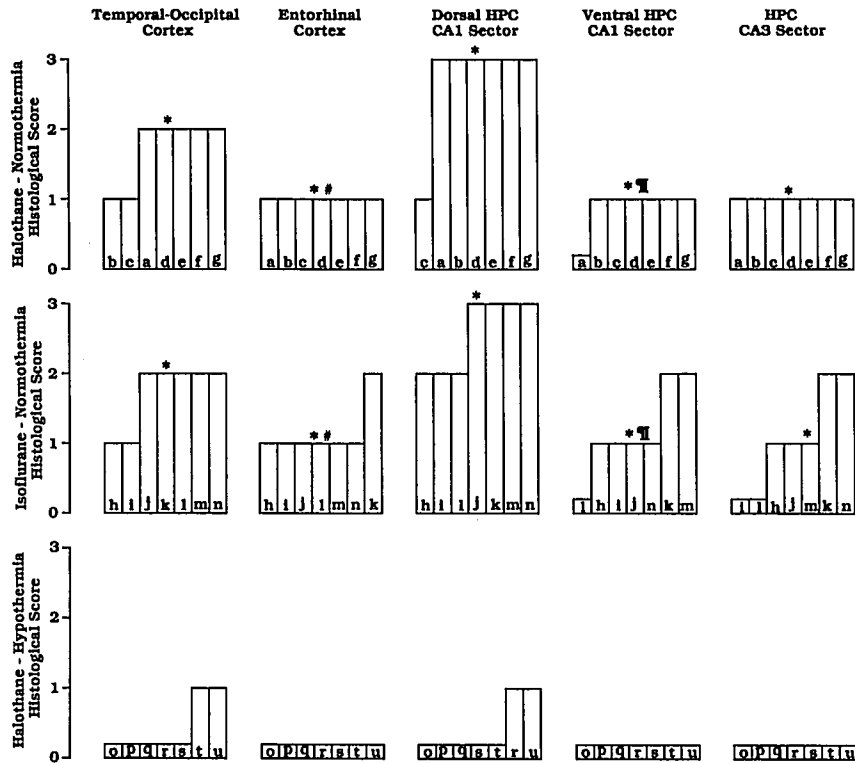


FIG. 3. Histologic grade in the caudal section (section c in fig. 1) in each of five structures in the three experimental groups: halothane-normothermia (top); isoflurane-normothermia (middle); and halothane-hypothermia (bottom). Each bar represents data for one animal, and each animal is identified by the same letter of the alphabet used in fig. 2. The grading scale is the same as that used in figure 2. *A significant difference ($P < 0.05$) for that structure between the halothane- or isoflurane-normothermia group and the halothane-hypothermia group. #An intragroup difference ($P < 0.05$) between temporal-occipital and entorhinal cortex. †An intragroup difference ($P < 0.05$) between the CA1 areas of dorsal and ventral hippocampus.

completed with either sodium nitroprusside added to an anesthetic of 0.75% halothane or with isoflurane alone ($1.9 \pm 0.7\%$ end-tidal). They found no differences in neurologic or histologic outcome. Baughman *et al.*⁸ compared the effects of 1.0 MAC isoflurane, 1.0 MAC halothane, and 70% nitrous oxide on neurologic and histologic outcome and mortality after incomplete cerebral ischemia induced by unilateral carotid artery occlusion with hemorrhagic hypotension in rats. All outcome measures were worse in the animals that were sedated with nitrous oxide than in those anesthetized with either halothane or isoflurane, but there were no differences between the animals that received halothane and isoflurane.⁸ Ruta *et al.* compared the extent of the area of histochemical injury (TTC) after a 4-h period of MCAO in rats anesthetized with 1.2 MAC concentrations of halothane or isoflurane and identified no differences.⁹ Warner *et al.* submitted rats to 2 h of MCAO during anesthesia with methohexital, isoflurane, or halothane.¹⁰ The former two were administered in concentrations sufficient to produce EEG burst suppression, and the halothane was administered in MAC concentrations equivalent to the isoflurane. Four days postischemia, infarct volume was smaller in the animals that received methohexital, but there were no differences between the isoflurane and halothane group animals.

By contrast, a laboratory investigation by Milde *et al.* suggested a protective effect of isoflurane.¹¹ Those investigators performed a 5-h period of MCAO in monkeys

anesthetized with isoflurane or thiopental to the point of EEG burst suppression. There were no differences in neurologic and histologic outcome between the groups, and, on the basis of previous demonstrations in similar paradigms of protection by barbiturates, they concluded that isoflurane had provided protection. However, the absence of a control group, either positive or negative, to confirm the sensitivity of the model has meant that this study too has been subject to criticism.

Isoflurane was ineffective as a cerebral protectant in the present investigation. The negative result, while consistent with the majority of previous studies, does not exclude the possibility of some protective efficacy. There are at least two possible explanations as to why isoflurane may have been ineffective in the present investigation: the use of an inadequate concentration of isoflurane, and/or evaluation in the context of an ischemic insult of excessive severity.

ISOFLURANE CONCENTRATION

Suppression of CMR is suspected to be at least part of the basis of the cerebral protective effects of anesthetic agents. The decrease in cerebral oxygen consumption with isoflurane is maximal at the point of EEG isoelectricity.¹ Although the 1.3 MAC concentration used in the present study was sufficient to cause an early EEG burst suppression pattern, complete isoelectricity does not occur

consistently below inspired concentrations of approximately 2 MAC in rats.¹² Accordingly, with 1.3 MAC isoflurane, CMR suppression was inevitably submaximal. Nonetheless, it was probably substantial. On the basis of the investigations by Maekawa *et al.*²² of the effect of isoflurane on the CMR for glucose in the rat, we estimate that CMR in parietal cortex and dorsal hippocampus in the isoflurane group in the present study were reduced by at least 56% and 37% respectively from unanesthetized control values. Furthermore, the 1.3 MAC concentration used in the present study was at least twice the average isoflurane MAC multiple reported as being administered at the time of carotid occlusion in a subset of the population⁴ of carotid endarterectomy patients in which Michenfelder *et al.* reported reduction of critical CBF.²¹ Despite substantial, albeit submaximal, suppression of CMR, cerebral protection was not apparent histologically in the present investigation.

SEVERITY OF ISCHEMIA

It has been suggested that anesthetics that reduce CMR are most likely to provide cerebral protection in circumstances in which the ischemic insult is insufficient to abolish electrophysiologic function completely, *i.e.*, circumstances in which there is some residual EEG activity.²³ Previous failures to demonstrate protection may have been the result of assessment in the setting of excessive degrees of ischemia. For that reason, this study was designed to assess the protective effects in brain regions that had sustained ischemic injuries of varying degrees of severity. EEG was not recorded. Others have reported that, in Wistar rats, the method used in the present study produces isoelectricity of the frontoparietal EEG,¹⁸ and it is likely that isoelectricity occurred over a portion of the forebrain in the present animals. However, it has been demonstrated with this method of inducing ischemia that there is a rostrocaudal gradient in the severity of the reduction of CBF, with more posterior regions having higher flows.²⁴ The coronal planes for histologic analysis were chosen to encompass caudal regions with insults of lesser severity, and the results confirm that, with respect to cortex and the CA1 region of hippocampus, this was accomplished. There was nonetheless no evidence of protection. The present data indicate that histopathologic damage was similar with isoflurane and halothane regardless of the severity of the ischemia. This does not exclude the possibility of protection by 1.3 MAC isoflurane but suggests that any protective effect may be relevant to an extremely narrow range of physiologic circumstances.

By contrast, mild hypothermia (3° C) markedly attenuated ischemic injury in both the rostral and caudal sections. This result is consistent with several previous investigations. Busto *et al.* observed that reduction of brain

temperature by 2–3° C conferred marked cerebral protection in the setting of ischemia induced by four-vessel occlusion in rats.¹³ Minamisawa *et al.* made similar observations. They observed that when skull temperature was reduced by 2° C during forebrain ischemia accomplished by the same method used in the present investigation, there was a substantial reduction of histologic damage.¹⁴

The mechanism of the protective effect of hypothermia is not well defined. Suppression of CMR almost certainly contributes, but more recently it has become apparent that reduction of neurotransmitter release also may be relevant.²⁵ It is also possible that hypothermia has specific influences on other elements of the cascade of biochemical events initiated by ischemia, *e.g.*, calcium kinetics, eicosanoid synthesis, or free radical formation. However, these possibilities have not been examined experimentally. With respect to CMR effects, it has been reported that hypothermia reduces the CMR for oxygen in nitrous oxide-sedated rats at a rate of approximately 5% per degree Centigrade within a temperature range of 37–22° C.²⁶ This suggests that the CMR reduction attributable to hypothermia in the present study may have been on the order of 15%. The relative effects of halothane and isoflurane on CMR (the latter greater than the former)^{8,27} are such that the relative excess of CMR depression in the isoflurane–normothermia group over the halothane–normothermia group was also at least 15%, yet no protection was observed in the isoflurane normothermia group. This argues that hypothermia's effect on global CMR is not the entire explanation for the protective effect.

However, measurements of global CMR mask differences in the manner in which hypothermia and anesthetic agents influence CMR. Anesthetic agents are believed to depress only the component of substrate utilization associated with electrophysiologic activity, whereas hypothermia causes proportional reduction in the rate of energy utilization associated with both electrophysiologic function and with the homeostatic functions required for maintenance of cellular integrity.²⁸ It may be that decreasing the latter is important in preventing irreversible loss of membrane integrity during periods of diminished supply of energy substrates. Once again, this cannot be the entire explanation because in the investigation of Busto *et al.*,¹⁵ mild hypothermia resulted in protection even though severe depletion of brain energy metabolites and lactate accumulation occurred.

Another possible explanation for hypothermia's efficacy was revealed by a subsequent study of Busto *et al.*,²⁵ who demonstrated that mild hypothermia (3° C) can completely inhibit the periischemic increase in extracellular glutamate concentration. Glutamate is believed to be a critical early component in the cascade of biochemical processes that mediate ischemic neuronal injury.²⁹ The effect of hypothermia on neurotransmitter release and on

other aspects of the biochemical processes relevant to cerebral ischemia will, no doubt, be the subject of intense investigation in the near future.

In summary, the present results indicate that in a model of incomplete forebrain ischemia in the rat, 1.3 MAC isoflurane had no cerebral protective effect relative to an equi-MAC halothane-anesthetized control state. This absence of protection was apparent in brain regions that had sustained ischemic insults of varying severity. By contrast, mild hypothermia (3° C) markedly attenuated ischemic neuronal injury during anesthesia with 1.3 MAC halothane. The present results do not provide substantiation for the cerebral protective properties attributed to isoflurane, although they do not exclude the possibility of efficacy when isoflurane is administered in other concentrations or in circumstances of ischemic insults of lesser degrees of severity than that used in the present study. The results also suggest that whatever the protective effects of clinically relevant concentrations of isoflurane, they may be quite modest by comparison with the effects of mild hypothermia. These results, if applicable in humans, suggest that the choice of volatile anesthetic agent may ultimately prove less relevant to protection of the brain against ischemic injury than the application of mild hypothermia.

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