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Halothane Reduces Focal Ischemic Injury in the Rat When Brain Temperature Is Controlled

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Background: Previous work has demonstrated that rats anesthetized with halothane during focal cerebral ischemia have better histologic and neurologic outcome than do rats undergoing the same insult when awake. The purpose of this experiment was to determine whether this difference persists when brain temperature is held similar in halothane-anesthetized and awake experimental groups.

Methods: Two ischemia experiments were performed. In both, the middle cerebral artery was occluded for 90 min. Temperature was monitored from a radiotelemetered thermistor implanted in the cerebral cortex. Four days after ischemia, infarct volume and neurologic function were assessed. In experiment 1, brain temperature was not controlled in awake rats. Temperature in rats anesthetized with halothane, approximately 1 minimum alveolar concentration, was regulated by servomechanism by surface heating or cooling to replicate the temperature profiles generated by awake animals. To address methodologic issues regarding infarct volume analysis, a subset of nine rats was examined for the effect of the histologic staining technique and the mathematical modeling algorithms used for computation of infarct volume values. In experiment 2, the brain temperature of awake and halothane-anesthetized rats was maintained normothermic (38.0°C) throughout ischemia and early recirculation.

Results: In experiment 1 no difference between groups was observed for cortical (halothane $146 \pm 95 \text{ mm}^3$ and awake $126 \pm 108 \text{ mm}^3$; $P = 0.64$) or subcortical (halothane $110 \pm 48 \text{ mm}^3$ and awake $100 \pm 66 \text{ mm}^3$; $P = 0.66$) infarct volume. Neurologic function was also similar between groups. Total infarct volume

was approximately 11% greater when histologic sections were stained with hematoxylin and eosin than when they were stained with nitro blue tetrazolium, although volumes correlated closely between the two techniques ($r^2 = 0.996$). Analysis by orthogonal or frustum projection from two-dimensional planimetric areas to three-dimensional volumes resulted in nearly identical values ($r^2 = 0.999$). In experiment 2, halothane-anesthetized rats experienced a 46% reduction in cortical infarct volume (halothane $106 \pm 97 \text{ mm}^3$ and awake $197 \pm 103 \text{ mm}^3$; $P = 0.03$). The incidence of hemiparesis was reduced in the anesthetized group ($P = 0.03$).

Conclusions: When brain temperature was maintained normothermic throughout the focal ischemic insult, a neurologic and histologic protective effect for halothane anesthesia was observed. This effect of halothane was not sufficient to persist when large variations in brain temperature were allowed. Regulation of brain temperature is a critical factor in the determination of the effects of anesthetics on focal ischemic brain damage. (Key words: Anesthetics, volatile; halothane. Animals: rat. Brain: ischemia; middle cerebral artery. Histology: hematoxylin and eosin; nitro blue tetrazolium. Measurement techniques: image analysis.)

VOLATILE anesthetic agents have long been thought to influence the pathophysiologic process of cerebral ischemia. Early work found infarct size resulting from middle cerebral artery occlusion (MCAO) to be greater in dogs anesthetized with halothane than in dogs undergoing the insult while awake or when anesthetized with pentobarbital.¹ Later work in a primate model of MCAO also found halothane-anesthetized animals to fare worse than did those anesthetized with pentobarbital.²

Therefore, interest in the use of volatile anesthetics as a strategy for neuroprotection waned until the advent of isoflurane. Given the substantially greater depression of cerebral metabolic rate and greater cerebral blood flow provided by isoflurane, it was proposed that this agent may favorably influence intras ischemic energy supply-and-demand relations and improve outcome.^{3,4} This, however, did not prove to be the case when the effects of isoflurane were compared with those of a variety of other anesthetic drugs in models of focal cerebral ischemia.⁵⁻⁷

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More recently, sevoflurane, having cerebral metabolic rate and cerebral blood flow responses similar to those of isoflurane,^{8,9} also was examined in a rat outcome model of temporary focal ischemia.¹⁰ In that study the effects of sevoflurane and halothane were found to be similar, but more importantly, animals anesthetized with either drug were observed to experience substantially less injury than those maintained awake. Although it is possible that anesthesia, regardless of the drug, provides a more favorable environment for an ischemic challenge than does the awake state,¹¹ it was suggested that the effects of the volatile anesthetics may be attributable to better control of brain temperature than in the awake state. To test this possibility, we performed the following experiments, in which cerebral temperature was held identical between awake and halothane-anesthetized rats undergoing temporary MCAO.

Materials and Methods

The following studies were approved by the Duke University Animal Care and Use committee.

Experiment 1

Male Wistar rats (age 8–10 weeks; body weight 250–300 g) were anesthetized with 40 mg/kg intraperitoneal sodium pentobarbital. Each animal was positioned in a stereotactic head frame with specialized ear bars designed to prevent damage to the tympanic membranes. Using aseptic technique, a midline scalp incision was made after local infiltration of the skin with 1% lidocaine. A burr hole was carefully drilled over the left hemisphere at bregma = 0 mm. A radiotelemetered thermistor (Brain Probe XM-FH, Mini Mitter, Sunriver, OR) was advanced into the cerebral cortex to a depth of approximately 2 mm. The probe was fixed in place with cyanoacrylate glue, and the wound was closed with suture. The animals were returned to their cages after recovery of the righting reflex. Before this procedure the temperature probe was calibrated in a circulating water bath against a mercury thermometer standard within the range of 35.0°C–40.0°C. This allowed extrapolation of temperatures from calibration points in accordance with the radiofrequency emitted by the thermistor.

Three to 5 days after the above procedure, rats were fasted but allowed free access to water for 12–16 h. Each rat was then anesthetized with 2–3% halothane in 40% O₂, balance nitrogen. After tracheal intubation

the lungs were mechanically ventilated to maintain normocapnia. The halothane concentration was reduced to 1.0–1.5%. By surgical incision, the tail artery was catheterized to monitor mean arterial blood pressure (MAP) and to sample blood. The animals were then prepared for MCAO by using modifications of the techniques described by Zea Longa *et al.*¹² and Memezawa *et al.*¹³ A midline cervical skin incision was made, and the right common carotid artery was identified. The external carotid artery was isolated, and the occipital, superior thyroid, and external maxillary arteries were ligated and divided. The internal carotid artery was then dissected distally until the origin of the pterygopalatine artery was visualized.

After surgical preparation, animals were randomly assigned to one of two groups. In one group (halothane, *n* = 12), rats remained anesthetized with 1.0–1.3% halothane. In a second group (awake, *n* = 12), halothane was continued until onset of ischemia and then abruptly discontinued.

After arterial blood gas and pH, plasma glucose, hematocrit, and MAP measurements, all rats underwent 90 min of MCAO. MCAO was achieved by introduction of a 0.25-mm-diameter nylon filament into the stump of the external carotid artery. The filament was passed approximately 23 mm distally through the internal carotid artery until slight resistance was felt.

The halothane group continued to receive 1.0–1.3% halothane throughout the 90-min ischemic interval. In the awake group, the wound was loosely closed by suturing, and halothane was discontinued. The endotracheal tube was removed upon resumption of adequate spontaneous ventilation. The interval between introduction of the filament and recovery of the righting reflex was typically 5–8 min. Rats in this group were placed in an methylmethacrylate polymer box containing 40% O₂/60% N₂.

During ischemia and the first 30 min of recirculation, cortical temperature was managed as follows. Analog signals from the radiotelemetered thermistor were received (Telemetry Receiver RA1010, Data Science, St. Paul, MN), converted to digital signals, and processed through a computer (4DX-33V, Gateway 2000, North Sioux City, SD). Each rat in the halothane group was assigned an awake rat. The awake rat was allowed to thermoregulate spontaneously. During the 90-min ischemic interval and the first 30 min of reperfusion, a temperature profile was generated and recorded with temperature sampled every 1 min. For the halothane-anesthetized rat, this profile was used as a template.

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Surface heating or cooling served to control the anesthetized rat's brain temperature to replicate the temperature profile generated by the awake rat.

MAP was measured continuously throughout the ischemic interval in both groups. Arterial blood gases and pH were measured 45 min after onset of MCAO.

After 90 min of MCAO, the filament was removed in the halothane group. Rats in the awake group were reanesthetized with halothane approximately 6 min before the conclusion of the ischemic interval. These spontaneously breathing rats continued to receive halothane through a snout cone during closure of surgical wounds. Fifteen minutes after the onset of recirculation, halothane was discontinued and all rats were allowed to awaken. In the halothane-anesthetized rats the trachea was extubated when spontaneous ventilation had resumed. All animals were then placed in 40% O₂/60% N₂ for 2 h and then were returned to cages with unrestricted access to food and water.

All animals were neurologically evaluated 96 h after reperfusion. Each rat was assigned a score from a scale of 0–3, in which 0 = no observable deficit; 1 = forelimb flexion; 2 = decreased resistance to lateral push without circling; and 3 = same behavior as 2, with circling.¹⁴ Neurologic tests were performed by a single observer blinded to the experimental condition of the animal.

After neurologic evaluation, animals were weighed and anesthetized with 4% halothane in O₂. They were then decapitated. The brains were removed and frozen at –20°C in 2-methylbutane. With use of a cryotome, quadruplicate 20- μ m-thick coronal sections were taken at 660- μ m intervals over the rostral-caudal extent of the infarct. The sections were dried and stained with hematoxylin and eosin. For each interval an additional quadruplicate set of sections was cut and stained for approximately 30 min with a solution containing 4.052 g sodium succinate and 0.135 g nitro blue tetrazolium (Sigma, St. Louis, MO) dissolved in 300 ml buffered distilled water (pH 7.2, 30°C).^{15,16} This process causes normal tissue to stain blue and infarcted tissue to appear white. The slides were then rinsed with saline, dehydrated in graded strengths of ethanol, and cleared with xylene.

Infarct volume was measured by digitally sampling sections stained with hematoxylin and eosin with a video camera (CCD XC711, Sony, Japan) controlled by a computer (Macintosh IIci, Apple, Cupertino, CA) using Image 1.54 software (National Institutes of

Health, Bethesda, MD). The image of each section was stored as a matrix of pixel units. For each tissue section, the pixel units were calibrated to give values as squared millimeters. The digitized image was then displayed on a video screen. With the observer blinded to experimental condition, infarct borders in the cortex and the subcortex were individually outlined (corpus callosum excluded) with an operator-controlled cursor. Infarct volumes (in cubic millimeters) were computed as running sums of infarct area multiplied by the known interval (e.g., 660 μ m) between sections over the extent of the infarct.

Physiologic values were compared between groups to preserve statistical power for analysis of major dependent variables. Infarct volumes were compared between groups by the unpaired Student's *t* test. Infarct volumes were correlated with neurologic grades by the Spearman rank correlation coefficient, and neurologic grades were compared between groups by the Mann-Whitney U test. The relation between inraischemic temperature and infarct size was compared by linear regression analysis. Parametric values are expressed as means \pm standard deviation.

Experiment 2

Preischemic placement of radiotelemetered thermistors, surgical preparation for and induction of ischemia, and recovery were performed as described for experiment 1. In experiment 2, again two groups were studied (halothane *n* = 13 and awake *n* = 13), and anesthesia was managed as in experiment 1.

Experiment 2 differed from experiment 1 only with respect to cortical temperature management. Before implantation each calibrated radiotelemetered thermistor was placed in a circulating water bath held at a constant temperature of 38.0°C. A template for the computerized servomechanism-controlled regulator was acquired with a duration of 3 h. This template served as the reference value for animals in both groups, allowing cortical temperature to be held at 38.0°C during the entire ischemic interval and the first 30 min of recirculation. If the value transmitted from the rat was less than 38.0°C, a heat lamp was automatically turned on; if brain temperature was greater than 38.0°C, chilled room air was blown over the surface of the animal by automated control of the gas source. Statistical analysis was as described for experiment 1.

Experiment 3

This experiment was performed to determine whether infarct volume measurement is dependent on

Table 1. Physiologic Values for Experiment 1 (Cortical Temperature Match Controlled Between the Two Groups)

	Halothane (n = 12)	Awake (n = 12)
2 min pre-ischemia		
MAP (mmHg)	71 ± 10	73 ± 11
pH _a	7.39 ± 0.06	7.39 ± 0.06
Pa _{CO₂} (mmHg)	39 ± 3	37 ± 2
Pa _{O₂} (mmHg)	167 ± 34	138 ± 24
Glucose (mg/dl)	143 ± 13	146 ± 20
Hematocrit (%)	42 ± 1	42 ± 2
45 min after onset of ischemia		
MAP (mmHg)	82 ± 11	118 ± 16
pH _a	7.35 ± 0.05	7.40 ± 0.02
Pa _{CO₂} (mmHg)	41 ± 4	39 ± 3
Pa _{O₂} (mmHg)	159 ± 28	156 ± 46
Cortical temperature (°C)		
T ₀	38.0 ± 0.1	38.0 ± 0.1
T ₃₀	37.1 ± 0.8	37.1 ± 0.8
T ₆₀	37.5 ± 1.0	37.4 ± 1.3

Values are mean ± SD. T₀, T₃₀, and T₆₀ refer to 0, 30, and 60 min, respectively, after onset of ischemia.

the staining technique used for frozen sections. Rats from experiment 1 were ranked according to total infarct volume. Every third rat was selected for this analysis, in which infarct volume was calculated (as described for experiment 1) based on images acquired from the sections stained with nitro blue tetrazolium. Infarct volumes as obtained from each rat using nitro blue tetrazolium and hematoxylin and eosin staining techniques were compared by linear regression analysis.

Experiment 4

This experiment was performed to determine whether infarct volume measurement is dependent on

the algorithm used for computation of volume from measured areas of infarct in serial sections with known distances between sections. Two methods for estimating infarct volumes were examined: (1) the standard method, in which the areas of infarction outlined in each of the serial tissue sections were assumed to project orthogonally from the plane of the section, and (2) a method based on the assumption that the region of infarction between neighboring tissue section formed a right-angle conical frustum. Every third rat from experiment 1 was selected for this analysis. Infarct areas were measured as described in experiment 1 using sections stained with hematoxylin and eosin. Infarct volumes were calculated from the area measurements using both the standard orthogonal projection volume formula and the right-angle frustum formula:

$$V_{\text{orthogonal}} = \sum [D_{i,i+1} (A_i + A_{i+1})/2]$$

$$V_{\text{frustum}} = \sum [\pi D_{i,i+1} (r_{i+1}^3 - r_i^3)/(r_{i+1} - r_i)/3],$$

where V = volume; D_{i,i+1} = distance between neighboring sections i and i + 1; A_i = measured infarct area for section i; and r_i = (A_i/π)^{1/2}.

Total infarct volumes (cortical plus subcortical infarct volumes) as calculated for each rat using the standard and frustum methods were compared by linear regression analysis.

Results

Experiment 1

Physiologic values (table 1) were similar between groups with the exception of MAP, which was greater at the midpoint of the ischemic interval in the awake group. Brain temperature varied considerably between

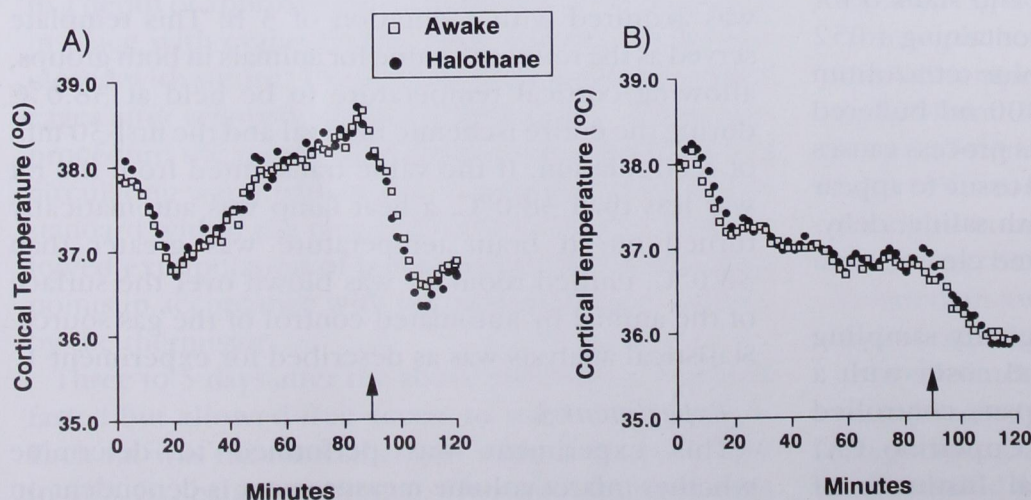


Fig. 1. Each panel (A and B) illustrates brain temperature profiles from two rats undergoing 90 min of temporary middle cerebral artery occlusion followed by 30 min of reperfusion. Open squares = values from an awake rat for which no attempt was made to control brain temperature; filled circles = values for a halothane-anesthetized rat for which surface heating or cooling was used to maintain brain temperature nearly identical to the temperature profile generated by the awake rat; arrows = onset of reperfusion.

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hypothermia and hyperthermia in the uncontrolled awake rats (fig. 1). Nevertheless, brain temperature values in halothane-anesthetized rats closely tracked the respective templates generated by the awake rats. Deviations of more than 0.2°C from the template were rare.

Neurologic grades were similar between groups (fig. 2). There was no difference between groups for cortical (halothane $146 \pm 95 \text{ mm}^3$ and awake $126 \pm 108 \text{ mm}^3$; $P = 0.64$) or subcortical (halothane $110 \pm 48 \text{ mm}^3$ and awake $100 \pm 66 \text{ mm}^3$; $P = 0.66$) infarct volumes although the coefficient of variation was large. A relation between brain temperature, as measured 45 min after onset of ischemia, and cortical ($r^2 = 0.661$; $P = 0.006$) or subcortical ($r^2 = 0.581$; $P = 0.0034$) infarct volumes was present. Neurologic grade correlated with total infarct volume in both groups ($P < 0.001$).

Experiment 2

Physiologic values (table 2) were similar between groups with the exception of MAP, which was greater at the midpoint of the ischemic interval in the awake group. The thermoregulation technique was successful in maintaining both awake and anesthetized brain temperature at $38.0 \pm 0.02^{\circ}\text{C}$ (fig. 3).

Neurologic grades were different between groups with a lower incidence of hemiparesis in animals receiving halothane during the ischemic insult ($P = 0.04$, fig. 2). Cortical infarct volumes (halothane $106 \pm 97 \text{ mm}^3$ and awake $197 \pm 103 \text{ mm}^3$; $P = 0.03$) were also reduced in rats anesthetized with halothane. Although a trend for reduced subcortical infarct volume was ob-

Table 2. Physiologic Values for Experiment 2 (Cortical Temperature Held at 38.0°C in Both Groups)

	Halothane (n = 13)	Awake (n = 13)
2 min pre-ischemia		
MAP (mmHg)	82 ± 15	76 ± 18
pH _a	7.37 ± 0.04	7.39 ± 0.05
Pa _{CO₂} (mmHg)	39 ± 3	38 ± 4
Pa _{O₂} (mmHg)	155 ± 23	151 ± 27
Glucose (mg/dl)	140 ± 10	136 ± 18
Hematocrit (%)	40 ± 3	41 ± 2
45 min after onset of ischemia		
MAP (mmHg)	85 ± 15	124 ± 11
pH _a	7.38 ± 0.05	7.44 ± 0.03
Pa _{CO₂} (mmHg)	41 ± 4	35 ± 3
Pa _{O₂} (mmHg)	142 ± 26	113 ± 27
Cortical temperature ($^{\circ}\text{C}$)		
T ₀	38.0 ± 0.1	38.0 ± 0.1
T ₃₀	38.0 ± 0.1	38.0 ± 0.1
T ₆₀	38.0 ± 0.1	38.0 ± 0.1

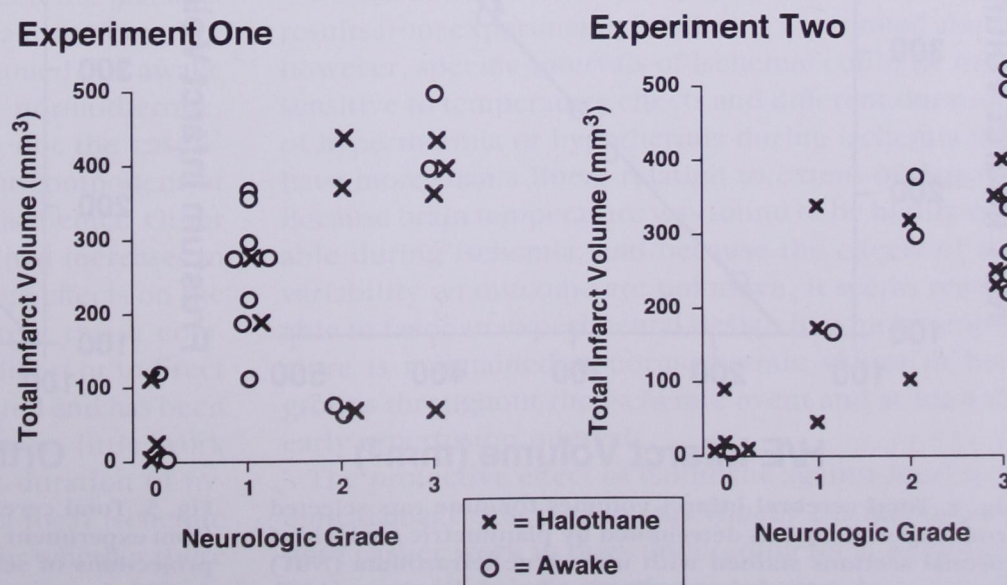
Values are mean \pm SD. T₀, T₃₀, and T₆₀ refer to 0, 30, and 60 min, respectively, after onset of ischemia.

served in halothane-anesthetized rats, it did not achieve significance (halothane $68 \pm 58 \text{ mm}^3$ and awake $100 \pm 49 \text{ mm}^3$; $P = 0.15$). Neurologic grade was found to correlate closely with infarct volume in both groups ($P = 0.004$).

Experiment 3

Infarct volumes determined from brain sections stained with hematoxylin and eosin or nitro blue tetrazolium were found to be strongly correlated ($r^2 =$

Fig. 2. Total cerebral infarct volume and neurologic grades (0 = no deficit) for individual awake and halothane-anesthetized rats in experiments 1 and 2.



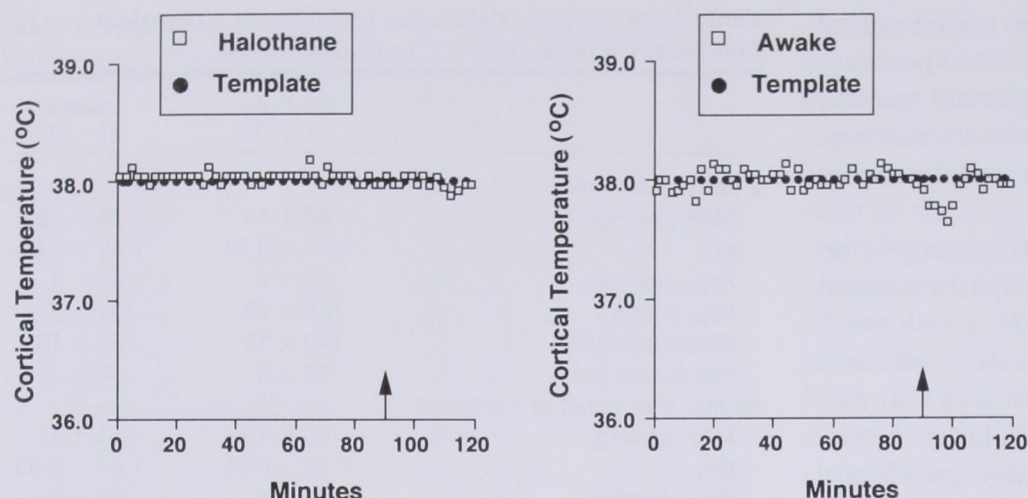


Fig. 3. Brain temperature (open squares) during 90 min of temporary middle cerebral artery occlusion followed by 30 min of reperfusion in an anesthetized rat (left) and in a rat undergoing ischemia while awake (right). Filled circles = target temperature of 38°C; arrows = onset of reperfusion.

0.99; $P < 0.0001$). Despite this correlation, infarct volumes were observed to be approximately 11% less when derived from sections stained with nitro blue tetrazolium (hematoxylin and eosin $324 \pm 112 \text{ mm}^3$ and nitro blue tetrazolium $287 \pm 124 \text{ mm}^3$) (fig. 4).

Experiment 4

Values for infarct volumes derived with the orthogonal projection model were consistently greater than those found with the conical frustum approximation, although the differences were small. Mean difference

\pm SD between the two estimates for the set of nine total infarcts examined was $5 \pm 1 \text{ mm}^3$ (range 3–6 mm^3). In no case did the two volume estimates differ by more than 2.2% (mean difference $1.4 \pm 0.5\%$). Regression analysis confirmed the strong correlation between the orthogonal and frustum estimates of infarct volume ($r^2 = 0.999$; $P < 0.0001$) (fig. 5).

Discussion

The potential exists for focal cerebral ischemic insults to occur during a variety of surgical procedures that

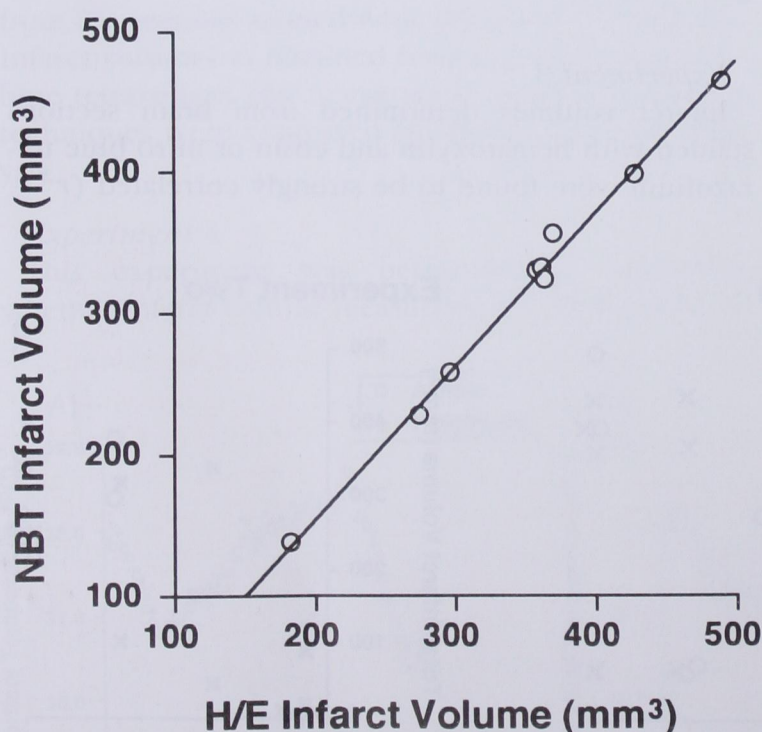


Fig. 4. Total cerebral infarct volumes for nine rats selected from experiment 1 as determined by planimetric analysis of coronal sections stained with nitro blue tetrazolium (NBT) versus hematoxylin and eosin (H/E). A best-fit line is provided.

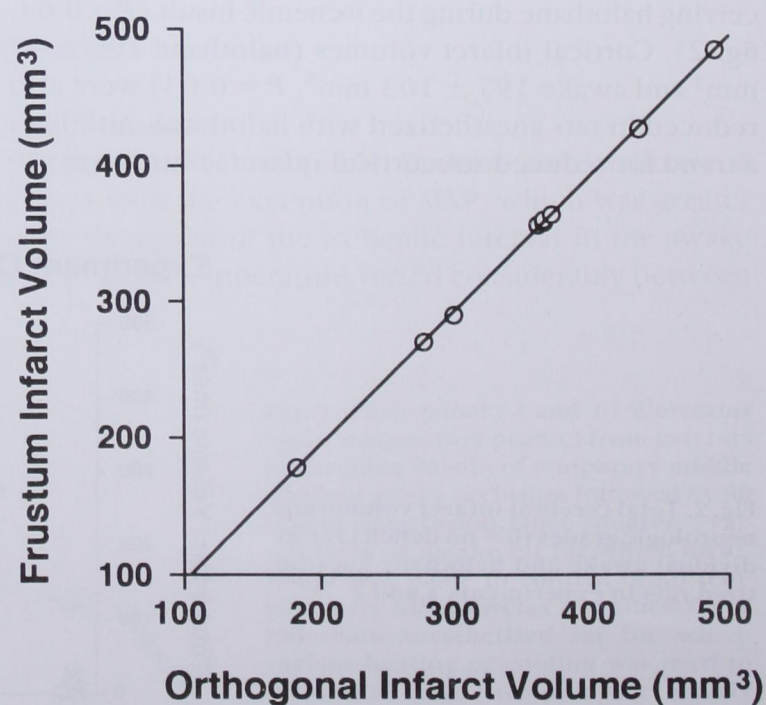


Fig. 5. Total cerebral infarct volumes for nine rats selected from experiment 1 as calculated by orthogonal versus frustum projections of serial coronal infarct areas. A best-fit line is provided.

require administration of anesthetic agents. Because anesthetic agents can have substantial effects on cerebrovascular and metabolic events, there is ample reason to define potential interactions between administered drugs and the pathophysiologic process of ischemia. Presumably, anesthetic agents have the potential to worsen or to improve outcome.

Many investigations in this area have compared the effects of one agent with those of another, for two reasons. First, it can be assumed that in most clinical situations some anesthetic is required, and thus the goal has been to identify the available drugs that have the greatest potential to reduce injury. Second, many animal models of focal ischemia are surgically invasive and thus require anesthetics to be administered during the ischemic insult to obliterate the pain associated with the procedure. One problem with this practice is that often little difference is observed among the effects of specific anesthetic agents on outcome. It may be concluded that neither agent provides neuroprotection, or in contrast, that both agents are equally protective. Use of an awake control group allows a distinction between these two conclusions to be drawn.

Recent development of the filament MCAO model has allowed renewed interest in comparing the effects of anesthetic agents with the awake state during the insult. This model offers a far less invasive approach to the middle cerebral artery than does temporal craniectomy. Awake rats tolerate the ischemic event well with respect to maintenance of hemodynamic and ventilatory stability. Relevant physiologic variables, including MAP, blood gases, and plasma glucose concentration, can be monitored and controlled.

One problem with this model has been the management of brain temperature. Unless brain temperature is measured directly, it can only be assumed that awake animals undergoing ischemia remain normothermic. Recent evidence suggests that this is not the case.¹⁷ Hyperthermia appears to be a frequent component of the physiologic response to cerebral ischemia. Other work has demonstrated that even minimal increases in brain temperature have potentially large effects on the magnitude of damage.^{10,18} To the extent that a compound reduces brain temperature by direct or indirect mechanisms, protection may be expected and has been observed.¹⁹ To our knowledge, however, little work has been done to define exactly what duration of hyperthermia (or hypothermia) during a focal ischemic insult is necessary to increase damage or whether there are critical periods during ischemia when an episode

of hyperthermia (or hypothermia) is most likely to be relevant.

These considerations have led to a debate regarding the appropriate conduct of neuroprotective drug evaluation studies when an awake untreated group serves as control. Particularly, the question has been raised as to whether it is more appropriate to maintain all animals normothermic during the ischemic insult or to render drug-treated animals mildly hyperthermic to match the control group's physiologic characteristics with respect to temperature and thereby leave only specific mechanisms of action of the compound to be evaluated as a difference between treated and control groups.²⁰

Accordingly we performed the above series of experiments with cortical brain temperature continuously monitored during the ischemic event and early reperfusion interval. To our surprise, brain temperature did not follow a standard pattern of mild hyperthermia during ischemia in all awake rats (experiment 1). Cortical temperature was found to increase, decrease, or vary between hypothermia and normothermia within the same animal over the course of the ischemic insult. Halothane-anesthetized rats were temperature-matched with these awake animals. No difference in severity of injury was observed between groups. In contrast, in experiment 2, cortical temperature in both groups was maintained at 38.0°C, which is the reported normothermic temperature of rat brain.²¹ In these conditions, halothane was found to reduce mean cortical and subcortical infarct volumes by 46% and 32% respectively. Clearly, experimental design has a critical effect on the conclusion drawn.

We do not have an explanation for the disparity of results from experiments 1 and 2. As mentioned above, however, specific intervals of ischemia could be more sensitive to temperature effects and different durations of hyperthermia or hypothermia during ischemia may have more than a linear relation to extent of damage. Because brain temperature was found to be highly variable during ischemia, and because the effects of this variability on outcome are unknown, it seems reasonable to favor an experimental design in which temperature is maintained at normothermic values in both groups throughout the ischemic event and at least the early reperfusion interval.

The protective effect of halothane against focal ischemic damage observed in experiment 2 is inconsistent with earlier work in dogs undergoing focal ischemia.¹ In that study, halothane administered in doses similar

to those used in our experiment resulted in no difference in infarct volume compared with that in animals sustaining the insult while awake. In contrast, 2.0% halothane anesthesia resulted in a worsened outcome even though MAP and rectal temperatures were held similar between groups. In our experiment, MAP was substantially decreased in the anesthetized group. Work in rodent focal ischemia models has indicated that improved perfusion pressure results in reduced injury.^{22,23} This information supports the prediction that hypotension should worsen damage, and had blood pressure been supported in our anesthetized group, the protective effect of halothane could have been even greater. Thus other undefined factors must account for the discrepant outcomes. However, as the current study indicates, experimental design with respect to management of brain temperature is critical in predicting the protective effects for halothane.

Experiment 3 was designed to address the importance of histologic staining technique in defining the margins of necrotic tissue. Although infarct margins can be reliably identified by application of the succinate dehydrogenase stain triphenyltetrazolium chloride to fresh tissue sections,^{24,25} recovery intervals of several days often result in liquefactive necrosis, which presents considerable difficulty in maintaining integrity of hemispheric borders necessary to define the area of damage. For this reason, we have incorporated the use of nitro blue tetrazolium, which stains for succinate dehydrogenase in frozen sections.¹⁵ However, no validation studies have been performed to support this practice. Results from this experiment indicate that nitro blue tetrazolium, although it underestimates the area of ischemic damage by approximately 11% relative to hematoxylin and eosin, provides a consistent estimate across a wide range of infarct sizes.

The image analysis method used to calculate infarct volumes reported in this study is based on a simplifying assumption regarding the shape of the infarct between sequential tissue sections: namely, that the areas of infarct observed in a two-dimensional section project orthogonally from the plane of that section to the next section. This method is used commonly for estimating infarct volume, although alternative, and presumably more accurate methods exist. Because, however, an infarct would rarely if ever be expected to satisfy the simplifying assumption, the standard method for determining volume used in this study clearly introduces a systematic error.

To characterize the magnitude of this error, we recalculated the infarct volumes by using an alternative model for delineating the infarct region between neighboring tissue sections, one that did not require that the infarct borders project orthogonally from the plane of the tissue section. Specifically, the infarct region between neighboring sections was assumed to take the form of a right-angle conical frustum and the total infarct volume computed as the sum of the frustum volumes over the extent of the infarct.

The volume estimate derived by using the orthogonal model was consistently greater than the volume calculated using the frustum model. However, the magnitude of the difference between the two estimates was very small. Thus, we believe that in the current study, the error introduced by the orthogonal method used to calculate volume had a negligible effect on estimates of infarct volume.

In conclusion, rats administered halothane during a reversible focal ischemic insult sustained substantially less damage than did those undergoing the insult while awake when brain temperature for both groups was maintained at normothermia. When intraischemic brain temperature in awake rats was allowed to vary, temperature-matched halothane-anesthetized rats had similar neurologic and histologic damage. The results confirm that halothane, compared with the awake state, has no adverse effect on ischemic outcome and indicate that this agent has a neuroprotective potential in specific conditions of normothermia.

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