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Ischemic Depolarization during Halothane-Nitrous Oxide and Isoflurane-Nitrous Oxide Anesthesia

An Examination of Cerebral Blood Flow Thresholds and Times to Depolarization

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Background: **Isoflurane–N_2O anesthesia (as compared with halothane–N_2O) reduces the cerebral blood flow (CBF) at which electroencephalographic changes occur in humans subjected to carotid occlusion. In contrast, no differences were seen in rats when cortical depolarization (instead of the electroencephalogram) was used as the ischemic marker during equi-MAC isoflurane–N_2O and halothane–N_2O anesthesia. To extend these findings, we used laser-Doppler flowmetry to continuously examine CBF (CBF_{LDF}) and attempted to better define the relation between CBF and the time to depolarization (as a measure of the rate of energy depletion after ischemia).**

Methods: **Cortical CBF_{LDF} was measured in normothermic, normocarbic rats, and the cortical direct-current potential was recorded using glass microelectrodes. Animals were anesthetized with 0.75 MAC halothane or 0.75 MAC isoflurane, both in 60% N_2O. After baseline recordings, both carotid arteries were occluded. Five minutes later mean arterial pressure was rapidly reduced to and held at target values of 50, 45, 40, 30 or 0 mmHg. This mean arterial pressure was maintained (and CBF_{LDF} was continually monitored) until depolarization occurred, or for a maximum of 20 min.**

Results: **CBF_{LDF} values before and after carotid occlusion (but before hypotension) were similar in the two groups. As intended, CBF_{LDF} decreased as postocclusion mean arterial pressure was reduced and the incidence of cortical depolarization increased. The delay until depolarization, defined as the interval between the moment CBF_{LDF} reached 25% of the preocclusion baseline until depolarization occurred, decreased as CBF_{LDF} was reduced. However, there were no intergroup differences except after a circulatory arrest (CBF = 0), where cortical depolarization was seen ≈30 s later in isoflurane–N_2O–anesthetized rats.**

Conclusions: **The CBF threshold for cortical depolarization as measured by laser-Doppler flowmetry did not differ significantly between halothane–N_2O– and isoflurane–N_2O–anesthetized rats. There were also no important differences in the times until depolarization, other than a small difference when flow = 0. If the time to depolarization is reflects the potential ischemic injury, the it is unlikely that isoflurane–N_2O conveys any protective advantage relative to halothane–N_2O. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: halothane; isoflurane. Brain: anoxic depolarization; blood flow; cerebral metabolic rate.)**

PROGRESSIVE reductions of cerebral blood flow (CBF) result in the sequential loss of electroencephalographic (EEG) activity, evoked potentials and, finally, tissue high energy phosphates.[1–5] This is quickly followed by the loss of normal transmembrane ion homeostasis, which is manifested *in vivo* by a rapid increase in extracellular potassium.[6–9] This can be detected with ion-selective electrodes, or by recording the sudden deflection of the direct-current (DC) potential in the cerebral cortex relative to an extracranial reference. This so-called anoxic or terminal depolarization serves as an excellent marker of severe tissue ischemia[8–11] and typically occurs at CBF values very similar to those associated with rapid cell death.

In a recent study in our laboratory, ischemic depolarization was examined in rats anesthetized with isoflurane–N_2O or halothane–N_2O.[12] Animals were subjected to controlled hypotension after bilateral common carotid artery occlusion, and CBF was measured after 10 min of ischemia. By combining these data with a measure of the cortical DC potential, it was possible

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to estimate the CBF values resulting in a loss of cellular ion homeostasis and to compare these values between the two anesthetics. It was concluded that the ischemic CBF threshold did not differ between the two anesthetics, despite lower cortical cerebral metabolic rates for glucose in isoflurane–N_2O–anesthetized rats.

There were two important limitations to the previous study. First, a true ischemic threshold was not measured because CBF was determined after the occurrence of anoxic depolarization. To measure threshold, CBF should be ideally measured before depolarization, something which requires continuous CBF monitoring throughout the ischemic period. Second, in other groups of rats, there was a small but statistically significant intergroup difference in the time interval between the onset of ischemia and the appearance of cortical depolarization when CBF = 0 or with presumably very low CBF values (although CBF was not measured in these groups). Specifically, depolarization occurred 30–90 s later with isoflurane–N_2O anesthesia than it did with halothane–N_2O. It is possible that this delay will increase—and that the difference between the anesthetics will also increase—as ischemic CBF increases. It is also possible that there is a range of ischemic CBF values were depolarization will be seen in halothane–N_2O– but not in isoflurane–N_2O–anesthetized animals. If so, it would argue that there is a CBF "window" with isoflurane–N_2O anesthesia where marked prolongation of depolarization times might exist—something that might imply clinically useful cerebral protection.

To resolve these questions and to extend the results of our earlier work, an experiment was designed in which the laser-Doppler flowmeter (LDF) was used to continuously record cortical CBF (CBF_{LDF}) during the entire interval between the onset of ischemic conditions and depolarization. In this way, it was possible to better examine the relation between CBF and the time to depolarization during halothane–N_2O and isoflurane–N_2O anesthesia.

Materials and Methods

All aspects of this study were approved by the University of Iowa Animal Care and Use Committee.

Male Sprague-Dawley rats, weighing 274–365 g, were fasted overnight, with free access to water. Anesthesia was induced in a plastic box with 4% halothane or isoflurane in oxygen. All incisions were subsequently made after tissue infiltration with 1% lidocaine. A tracheotomy was performed, and mechanical ventilation was started (tidal volume $\approx$ 3.5 ml, respiratory rate 40–50 breaths/min). Anesthesia was thereafter maintained with $\approx$1–1.25 MAC of inhalation anesthetic in 40% O_2–balance N_2O. Halothane MAC was taken as 1.0–1.1%,[13,14] and isoflurane MAC was considered to be 1.4%.[13] Inspired concentrations of inhalation anesthetic were measured (Anesthetic Agent Monitor 222, Puritan Bennett, Wilmington, MA). Muscle paralysis was achieved with 0.6 mg *d*-tubocurarine chloride given subcutaneously. A rectal thermistor was placed and temperature was maintained at 37.5 ± 0.5°C with a warming blanket.

One femoral vein and two femoral arteries were cannulated for drug or fluid administration, for continuous blood pressure measurement, and for blood withdrawal during controlled hemorrhagic hypotension. The right and left common carotid arteries were then isolated and encircled with silk thread. Both ends of the thread were passed through a piece of plastic tubing to create a snare that would later be used for carotid occlusion. Arterial blood gases were measured at regular intervals and ventilation was adjusted to maintain arterial CO_2 tension at $\approx$38 mmHg and arterial O_2 tension > 100 mmHg. Mean arterial pressure (MAP) was kept >80 mmHg with 6% hetastarch (Hespan, DuPont, Wilmington, DE) given intravenously if necessary.

The animal was then turned prone and the head was fixed in a stereotactic frame (David Kopf Instruments, Tujunga, CA). The scalp was reflected and, working under a surgical microscope, a small frontal craniectomy (2 × 3 mm) was created with a high-speed electric drill. During drilling, the craniectomy site was irrigated with cool saline to avoid thermal injury to the cortex. Care was taken to leave the dura intact. After the craniectomy was complete, a needle thermistor (524, Yellow Springs Instruments, Yellow Springs, OH) was inserted under the periosteum lateral to the hole, and pericranial temperature was maintained at 37.5 ± 0.5°C, using a continuous stream of air which was passed through a heated humidifier (Cascade, Puritan Bennett). The inspired concentration of volatile anesthetic was then reduced to 0.75 MAC (0.8% halothane or 1.1% isoflurane), combined with 60% N_2O.

Direct-current Potential Recording

With the aid of a microscope, a saline-filled glass micropipette (tip diameter 2–5 μm) containing a Ag–AgCl wire electrode was inserted approximately 200 μm into the cortex, using a micromanipulator. Dural and pial blood vessels were carefully avoided. The mi-

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croelectrode was referenced to a Ag–AgCl disc electrode (Red Dot, 3M, Minneapolis, MN) placed on the shaved skin of the animal's back. The potential difference between the two electrodes was measured with an amplifier (7P122, Grass Instruments, Quincy, MA) equipped with a high-impedance input unit (H1P5, Grass). The signal was recorded on paper using a polygraph (79, Grass).

Laser-Doppler Cerebral Blood Flow Measurement

CBF was monitored by LDF (BPM 403A Blood Perfusion Monitor, Vasomedics, St. Paul, MN). With the aid of a microscope and using a micromanipulator, an LDF probe with an outer diameter of 0.8 mm was positioned as close to the Ag–AgCl microelectrode as possible. The probe was placed perpendicular to the dura without indenting it; large dural or pial blood vessels were avoided. The craniectomy site was slowly and continuously rinsed with saline to avoid drying of the dura and accumulation of blood under the probe. The CBF_{LDF} signal was recorded on paper *via* an amplifier (7P122, Grass). An averaging time of 0.5 s was selected. This setting allowed easy determination of CBF_{LDF} values, without obliterating any moment-to-moment variations of the waveform. Before placing the probe on the dura, a zero flow value was determined, using a zero flow signal from the instrument. A copper-screen Faraday cage was placed around the animal to minimize electrical noise. Fifty units of heparin were administered intravenously. Total preparation time was approximately 60 min (from induction of anesthesia to the start of DC potential and CBF_{LDF} monitoring).

Values obtained by LDF are designated CBF_{LDF}. The Vasomedics unit displays flow as "microvascular $ml \cdot 100\ g^{-1} \cdot min^{-1}$", but we prefer to report CBF_{LDF} values without specific units and as a fraction of baseline CBF (CBF before carotid artery occlusion).

Protocol

The basic protocol involved rapidly occluding both common carotid arteries, followed by the controlled reduction of MAP to one of several predetermined values. Initially, for each anesthetic, animals were randomly assigned to one of four blood pressure groups: 0 mmHg (halothane: n = 4, isoflurane: n = 4), 30 mmHg (n = 4 each), 40 mmHg (n = 8 each) and 50 mmHg (n = 8 each). After inspection of the results, an additional 16 animals were studied at MAP = 45 mmHg (n = 8 each) and 8 more rats were studied at MAP = 50 mmHg (n = 4 each). Overall, 72 rats were studied, 36 with each anesthetic.

In each animal, DC potential and CBF_{LDF} were monitored continuously. Thirty minutes after positioning the LDF probe on the dura, the left and right common carotid arteries were both quickly occluded. Five minutes later, hypotension was induced. In the 0 mmHg groups, 1 ml of saturated KCl was given intravenously. Return of the CBF_{LDF} signal to zero and the occurrence of anoxic depolarization were verified. In the 30-, 40-, 45-, and 50-mmHg groups, the selected MAP was obtained by opening the left femoral artery catheter to a saline-filled reservoir suspended at a predetermined height above the animal. This method made it possible to maintain MAP at the desired value ± 2 mmHg. The selected blood pressure was maintained until anoxic depolarization occurred or, if depolarization did not occur, for a maximum of 20 min. The animal was then killed with 1 ml of saturated KCl injected intravenously and return of the CBF_{LDF} signal to the zero flow mark was verified. If no depolarization was observed during hypotension, the appropriate functioning of the Ag–AgCl electrode was verified by observing terminal depolarization after KCl administration.

Data Analysis

CBF_{LDF} was recorded continuously on the polygraph. To simplify data analysis, CBF_{LDF} values were excerpted at 1 min intervals. Baseline CBF_{LDF} was calculated as the average of the CBF_{LDF} values obtained during a 20-min period before carotid artery occlusion. CBF_{LDF} during ischemia was defined as the average of CBF_{LDF} values recorded from the moment MAP reached the predetermined value until depolarization occurred (or for a maximum of 20 min if depolarization did not occur). CBF_{LDF} during ischemia was expressed both as an absolute (but unitless) value and as a percentage of the Baseline CBF_{LDF} value. The delay to depolarization was defined as the time (in seconds) from the moment CBF_{LDF} reached 25% of baseline until a fast negative DC potential shift occurred.

This approach to defining the delay to depolarization was chosen after completion of the study and examination of the data. It was obviously necessary to define a distinct and reproducible starting point for the calculation of the delay until depolarization. Many such "time zero" values could have been chosen, and many different intervals might have been calculated. However, because ischemic conditions are dependent on CBF (and not on such indirect factors as MAP) we

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thought that a starting point based on the achievement of ischemic flow conditions would be most appropriate. After considering several possibilities, we selected the moment at which CBF_{LDF} reached 25% of baseline as the starting point. Although this choice was somewhat arbitrary (we could also have chosen CBF_{LDF} = 50% or CBF_{LDF} = 20%, *etc.*), this value is close to the overall "threshold" for depolarization, *i.e.,* the highest CBF_{LDF} value in the experiment at which depolarization occurred was ≈24% of baseline. In addition, because the typical oscillations in the CBF_{LDF} tracing disappeared with the onset of hemorrhage, this point was easily marked. We also defined predepolarization CBF as the average of all values (taken every minute) between the point at which the target MAP was achieved and the onset of depolarization. Alternatively, we could have used a single value just before depolarization. However, CBF_{LDF} was remarkably stable after target MAP values were reached, and our results are unchanged if depolarization delays are plotted against this single value as opposed to the averaged CBF_{LDF} number reported.

Cerebral Blood Flow Measurement by the Indicator–Fractionation Method ([^{3}H]-Nicotine)

Because LDF does not measure absolute CBF, it is important to define the relation between absolute cortical CBF (in $ml \cdot min^{-1} \cdot 100\ g^{-1}$) and CBF_{LDF} and to confirm that, for example, a CBF_{LDF} value of 110 units represented equivalent actual CBF values with the two anesthetics. Absolute CBF was therefore measured by the indicator–fractionation method using [^{3}H]-nicotine as a tracer ($CBF_{[^3H]}$). This was done in a separate group of 10 normotensive animals (n = 5 for each anesthetic) and in 4 animals whose MAP was reduced to 40 mmHg (n = 2 for each anesthetic). Both carotid arteries were isolated in each rat but were not occluded. Surgical preparation was as described above except that a second femoral vein was cannulated for isotope infusion, and the second femoral artery was used for simultaneous withdrawal of a reference arterial blood sample. DC potential was not monitored, but CBF_{LDF} was determined.

Thirty minutes after placement of the LDF probe, and after MAP was stable, CBF was measured at 0.75 MAC of inhalation anesthetic–60% N_2O. [^{3}H]-Nicotine (25 μCi) in 0.5 ml saline was infused intravenously over 40 s. Simultaneously, a reference sample was withdrawn from the femoral arterial catheter into a preweighed syringe at a rate of 0.75 ml/min. As soon as the isotope infusion was completed, the animal was killed by intravenous injection of 1 ml of saturated KCl. The probe for LDF was immediately removed and the brain at the craniectomy site was frozen by pouring liquid nitrogen over the skull. A cortical brain sample of approximately 2 × 6 mm (10–20 mg) was then removed *via* a slightly enlarged craniectomy. All underlying white matter was carefully discarded. The sample was placed on a preweighed cover slip, weighed and placed in a 20-ml scintillation vial. One milliliter of tissue solubilizer (TS-1, Research Products International, Mt. Prospect, IL) was added. The vial was capped and then heated overnight at 50°C. Samples were neutralized with glacial acetic acid, after which 10 ml of 4a20 scintillation cocktail (Research Products International) were added.

After adding 500 U of heparin to the syringe, the arterial reference sample was weighed. Five 50 μl aliquots of the reference sample were then pipetted into scintillation vials and 0.6 ml of TS-1 was added to each. After 20 min incubation at 50°C, the samples were decolorized with 200 μl benzoyl peroxide and again heated at 50°C. After 30 min, glacial acetic acid and 10 ml of 4a20 scintillation cocktail were added and the vials were then kept overnight at 50°C.

The samples were protected from light for 3 days before counting in a Liquid Scintillation Analyzer (1900 TR, Packard Instrument Company, Downers Grove, IL). The [^{3}H] disintegrations per minute were determined in the reference sample and in the brain sample, and $CBF_{[^3H]}$ was calculated using the following equation:

$$CBF_{[^3H]} = \frac{\text{ref syringe flow} \times \text{brain tissue } [^3\text{H}]\text{ dpm})/\text{ref syringe } [^3\text{H}]\text{ dpm}}{\text{brain tissue weight}}$$

where ref = reference and dpm = depurations per minute.

Statistics

The relation between CBF_{LDF} and $CBF_{[^3H]}$ was examined by regression analysis. Because repeated measurements in our laboratory have shown that CBF_{LDF} values return to zero after a cardiac arrest (a condition during which no isotope can reach the brain), the regression lines were "forced" through the 0,0 point on the graph. Analysis of covariance was used to compare the isoflurane–N_2O and halothane–N_2O regression lines.

Physiologic variables measured during the baseline period (and for CBF_{LDF} after carotid occlusion) were compared between anesthetics using an unpaired Stu-

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dent's t test. A P value < 0.05 was considered significant.

During ischemia, each animal yielded four primary data items: MAP, CBF_{LDF}, the presence or absence of depolarization, and the time to depolarization. The effect of MAP on the incidence of depolarization was examined using a contingency table (chi-squared). Comparisons between depolarization incidences in the two anesthetic groups at any single MAP value was performed using Fisher's exact test. To examine the incidence of depolarization at different CBF_{LDF} values, CBF_{LDF} values measured during ischemia were combined into bins. For absolute CBF_{LDF} values, these bins were 0, 1–10, 11–15, >15 flow units (there were an insufficient number of data points to permit the use of a 1–5 bin). For relative flow values, CBF_{LDF} was expressed as a percentage of baseline CBF_{LDF}, and combined into the following bins: 0%, >0–10%, >10–15%, >15%. Contingency table analysis was again used.

To examine the times to depolarization, a scattergram plot of CBF_{LDF} *versus* time to depolarization was inspected. Although it was possible to draw lines through the points (*e.g.*, with Lowess), we were unable to find any data transformation or acceptable curve fit that would allow intergroup comparisons of the entire data set. Therefore, absolute CBF_{LDF} values were again combined into four bins: 0, 1–10, 11–15, and >15, and times to depolarization examined with a two-factor analysis of variance. In addition, a similar analysis was performed using CBF_{LDF} expressed as a percentage of baseline.

All statistical tests were performed using the Statview II or SuperANOVA programs (Abacus Concepts, Berkeley, CA) for the Macintosh (Apple, Cupertino, CA) computer.

Results

[^{3}H]-Nicotine Cerebral Blood Flow

The scattergram of paired CBF_{LDF} and $CBF_{[^3H]}$ values is shown in figure 1. A linear regression analysis was highly significant, with an r value for both lines of >0.89 ($P < 0.0001$). Analysis of covariance indicated no significant differences between the regression lines for the two anesthetic groups. It is thus reasonable to conclude that equal CBF_{LDF} values for the two anesthetics represented equivalent actual CBF values.

Laser-Doppler Cerebral Blood Flow

MAP, pericranial temperature, arterial blood gases, hematocrit and plasma glucose values measured during baseline conditions, as well as CBF_{LDF} values measured before and after bilateral carotid artery occlusion (but before hypotension) are shown in table 1. Although MAP and plasma glucose were significantly greater in animals anesthetized with isoflurane–N_2O, the differences were small. There were no other intergroup differences.

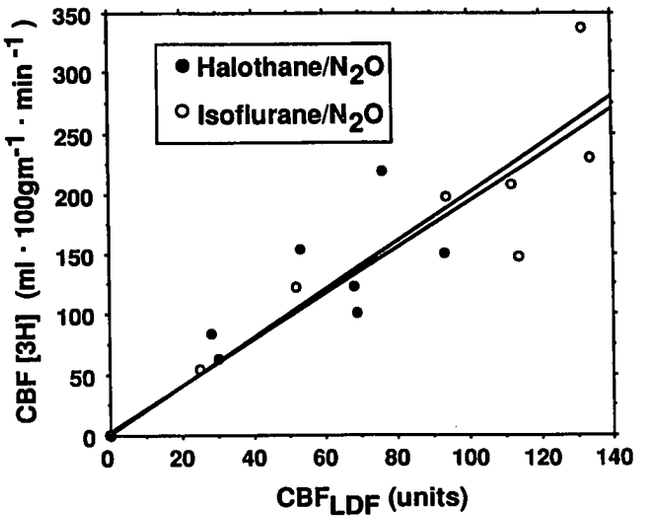

Fig. 1. A regression plot of cerebral blood flow measured by laser-Doppler flowmetry *versus* cerebral blood flow measured by the indicator–fractionation method with [^{3}H]-nicotine. There is a highly significant linear relation for both halothane–N_2O and isoflurane–N_2O ($r \geq 0.9$; $P < 0.0004$). Because repeated measurements confirm that a cardiac arrest leads to zero-flow values with both methods, the regression lines have been "forced" through zero.

Absolute CBF_{LDF} values and the incidence of depolarization in the various MAP groups were similar for halothane–N_2O– and isoflurane–N_2O–anesthetized animals (table 2).

The relations between absolute CBF_{LDF} and CBF_{LDF} expressed as a percentage of baseline and the incidence of depolarization for the two anesthetic groups is shown in table 3. There were no apparent intergroup differences.

Time to Depolarization

A scattergram of the relation between CBF_{LDF} and the delay until depolarization is shown in figure 2. As expected, the delay until depolarization increased with increasing CBF_{LDF} values. This was most notable in the lower flow ranges. Although statistical analysis could not be carried out on this type of data presentation, the evident scatter suggests that there are no important dif-

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Table 1. Baseline Physiologic Parameters

	Halothane/N_2O (n = 36)	Isoflurane/N_2O (n = 36)
Weight (g)	319 ± 15	318 ± 20
MAP (mmHg)	93 ± 11	104 ± 15*
Temperature		
Rectal (°C)	37.6 ± 0.3	37.7 ± 0.3
Pericranial (°C)	37.5 ± 0.2	37.6 ± 0.2
Pa_{CO_2} (mmHg)	38.7 ± 1.9	40.0 ± 2.2
Pa_{O_2} (mmHg)	123 ± 17	121 ± 14
*p*H	7.38 ± 0.02	7.37 ± 0.02
Hematocrit (vol %)	41 ± 2	42 ± 2
Blood glucose (mg/dl)	152 ± 32	196 ± 40*
Baseline CBF_{LDF} (no units)	87 ± 24	95 ± 25
CBF_{LDF} carotid occlusion		
Absolute values (no units)	56 ± 31	58 ± 38
% of baseline	65 ± 29	61 ± 35

Values are mean ± SD. Except for "CBF_{LDF} carotid occlusion," all data were recorded just prior to carotid artery occlusion.

* Significant difference between the two anesthetic groups ($P < 0.01$) as determined by unpaired *t* test.

Table 3. Depolarization Incidences *Versus* Absolute CBF_{LDF} and CBF_{LDF} as a Percentage of Baseline CBF_{LDF}

CBF	Halothane/N_2O [no. of depolarizations/ total (%)]	Isoflurane/N_2O [no. of depolarizations/ total (%)]
Absolute (units)		
0	4/4 (100%)	4/4 (100%)
1–10	11/12 (92%)	11/11 (100%)
11–15	11/14 (79%)	12/15 (80%)
>15	3/6 (50%)	3/6 (50%)
% of Baseline		
0%	4/4 (100%)	4/4 (100%)
>0–10%	5/5 (100%)	7/7 (100%)
>10–15%	12/13 (92%)	14/15 (93%)
>15%	8/14 (57%)	5/10 (50%)

There were no intergroup differences.

ferences between anesthetics. For further analysis, CBF_{LDF} was combined into bins, and plotted against the time to depolarization for the two anesthetics (animals in which depolarization did not occur are not included in this analysis). This is shown in figure 3. Although there was a significantly longer delay to depolarization in isoflurane–N_2O animals subjected to a cardiac arrest (CBF = 0), there were no differences at higher flows.

Discussion

It has been suggested that isoflurane has cerebral protective properties. This arises from the drug's ability (in clinically relevant doses) to produce profound EEG suppression and to decrease cerebral metabolic rate more than halothane.[15,16] Isoflurane in high doses can also slow the depletion of high energy phosphates during severe hypotension.[17] Unfortunately, efforts to show improved histopathologic or functional outcome in after global and focal cerebral ischemia have been generally unsuccessful.[18–23] Baughman *et al.* did show improved neurologic outcome after incomplete ischemia in rats anesthetized with isoflurane when compared with N_2O alone,[24] but this did not differ from the outcome seen with halothane. Milde *et al.* were unable to demonstrate differences in neurologic outcome and histopathology between isoflurane- and thiopental-treated groups in a primate model of focal ischemia.[25] However, the high incidence of severe stroke in both groups may have made it difficult to detect any differences.

This failure to demonstrate protection suggests that isoflurane has no advantages over other volatile agents. It also suggests that drug-induced metabolic suppression is insufficient to predict protective efficacy of an anesthetic.[26] However, in 1987, Messick *et al.* showed that CBF values associated with ischemia-induced EEG changes (the "critical CBF") during carotid endarter-

Table 2. CBF_{LDF} and Depolarization Incidences in the Five MAP Groups

	Halothane/N_2O			Isoflurane/N_2O		
BP Group	n	CBF_{LDF} (units)	No. of Depolarizations (%)	n	CBF_{LDF} (units)	No. of Depolarizations (%)
0	4	0	4 (100%)	4	0	4 (100%)
30	4	6 ± 2	4 (100%)	4	11 ± 6	4 (100%)
40	8	12 ± 2	8 (100%)	8	10 ± 3	8 (100%)
45	8	13 ± 3	7 (88%)	8	12 ± 4	7 (88%)
50	12	16 ± 9	6 (50%)	12	16 ± 7	7 (58%)

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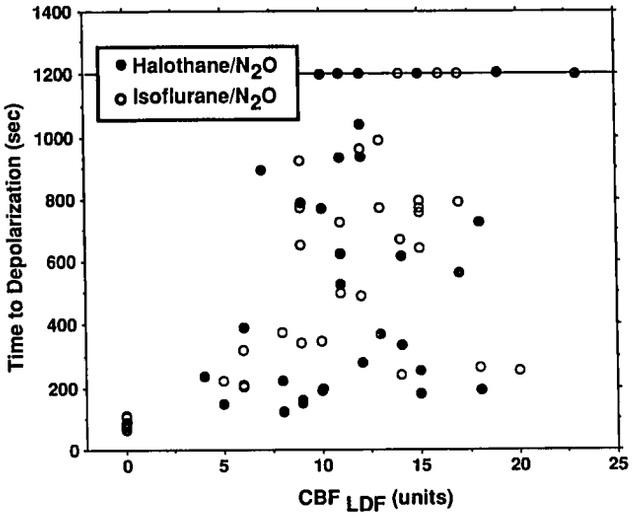

Fig. 2. Scattergram plot of cerebral blood flow measured by laser-Doppler flowmetry (CBF_{LDF}) *versus* time to depolarization. Each point represents one rat. Because the study was terminated at 1200 s of ischemia, the values lying on the horizontal line at 1200 s represent those animals in which depolarization did not occur. Two data points (one for halothane and one for isoflurane) representing CBF_{LDF} values >25 are not shown. Although no statistical analysis was performed, there is a clear (but widely scattered) increase in time to depolarization with increasing CBF_{LDF}, without evident intergroup differences. The mean ± SD values in groups in which mean arterial pressure = 0 were 78 ± 12 s (halothane–N_2O) and 103 ± 10 s (isoflurane–N_2O).

ectomy were lower during isoflurane–N_2O anesthesia than with halothane–N_2O.[27] In addition, both Blume *et al.*[28] and Michenfelder *et al.*[29] presented retrospective data suggesting that the incidence of "ischemic" EEG changes was lower in isoflurane–N_2O–anesthetized patients. It is thus possible that isoflurane might reduce at least one ischemic CBF threshold, and thereby provide clinically useful protection. However, neither study provided data showing a clinical benefit (as measured by outcome). It was this discrepancy (*i.e.*, reduced critical CBF but no clinical benefit) that prompted our earlier study. In that work, the CBF thresholds for both EEG change and cortical depolarization were studied in rats anesthetized with clinically relevant concentrations of isoflurane or halothane combined with N_2O.[12] It was concluded that these thresholds did not differ between the two anesthetics.

In this earlier study we monitored cortical DC voltage during controlled global ischemia, and measured CBF after 10 min of ischemia. Thus, CBF was measured after depolarization occurred. The median CBF value associated with depolarization was 10 ml · 100 g^{-1} · min^{-1} during 0.75 MAC halothane–60% N_2O anesthesia compared with 9 ml · 100 g^{-1} · min^{-1} during isoflurane–N_2O; *i.e.*, it was impossible to demonstrate any difference in the "critical CBF" for depolarization between these two anesthetics. However, when similarly anesthetized rats were subjected to a cardiac arrest (CBF = 0), the delay until depolarization was about 30 s longer in isoflurane–N_2O animals, a finding which was in keeping with the lower cortical cerebral metabolic rates for glucose in these animals.

This previous study raised two important questions. First, we did not, strictly speaking, measure an ischemic CBF threshold, because flow was recorded only after the onset of the cortical voltage shift. Ideally, a threshold flow value would be that recorded during the period before depolarization. In addition, we had ignored the delay until depolarization except in the very low-flow animals. It is possible that isoflurane "protection" might be seen as a prolongation of depolarization times, even if there were no difference in the absolute CBF thresholds for depolarization. In other words, it is possible that in spite of identical depolarization thresholds, one anesthetic might prolong the time until the onset of membrane failure long enough to provide practically useful protection. The 30 s delay seen during a cardiac arrest is unlikely to be of any clinical value, but because the time to depolarization should be longer at higher

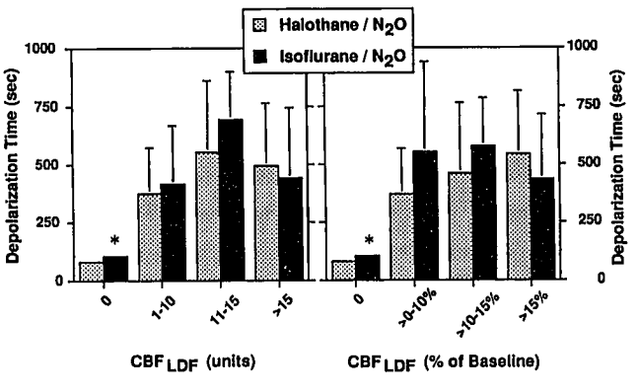

Fig. 3. A bar graph of cerebral blood flow measured by laser-Doppler flowmetry (CBF_{LDF}) and CBF as a percentage of baseline *versus* time to depolarization. CBF_{LDF} values are combined into bins of 0, 1–10, 11–15, and >15 units. CBF as a percentage of baseline values are combined into bins of 0%, >0–10%, >10–15%, and >15%. Delay values are means ± SD (except for the CBF = 0 group, in which the SD bars are too small to show; these SD values can be found in the legend for fig. 2). There were no significant differences in depolarization time except at the 0 flow bins (*$P < 0.05$ for halothane–N_2O *vs.* isoflurane–N_2O).

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flows,[12] there might be a range of CBF values within which the time to depolarization might be prolonged for many minutes (or perhaps indefinitely).

The present study was undertaken to examine CBF thresholds, with CBF measured by LDF before depolarization. The major limitation of this work concerns the use of the LDF to monitor CBF. LDF was introduced by Stern *et al.* in 1977[30] and was subsequently used to measure changes in CBF by Skarphedinsson *et al.* in 1988.[31] Validation studies have indicated that LDF is an excellent monitor of flow in the cortex and also accurately reflects flow changes during ischemia.[31–35] This method has become a standard for assessing acute changes in cortical CBF in rats due to factors such as drugs, physiologic interventions, regional electrical stimulation, and stroke. However, the current study did not use LDF to measure changes in CBF, but rather to compare continuously measured CBF between two groups of animals. Some might argue that because the numerical values generated by the LDF unit do not accurately reflect absolute CBF values, the device should not be used for such purposes. This seems to be an unreasonable criticism. There is no doubt that LDF provides a measure of cortical flow in a superficial tissue compartment. The resultant numerical values are normally distributed around a consistent mean value (although this value is not equal to that measured with standard methods), and the variance around that mean is comparable to that obtained using other flow techniques. This study confirms the work of others[32] that have shown a reasonable (although not absolute) correlation between LDF-measured flows and CBF measured by standard methods. Therefore, although the reported values derived by LDF cannot be directly converted into absolute flows, the comparisons of CBF_{LDF} values between the two anesthetic groups and between blood pressure groups should be valid. There is also no other widely accepted method that will allow the continuous monitoring of CBF over time before an event such as depolarization.

Why does isoflurane result in a significant prolongation of the time to depolarization when CBF = 0, but not when flow is higher? We believe that the answer is due to a combination of factors. The first is statistical. Under zero flow conditions, the times to depolarization are remarkably uniform; *i.e.*, the coefficient of variation (coefficient of variation = standard deviation/mean) is on the order of 10%. This makes it relatively easy to detect even tiny differences in depolarization times. However, as CBF increases, not only does the delay until depolarization increase (as in fig. 2), but the animal-to-animal variability increases enormously. For example, when CBF_{LDF} was in the range of 1–10 units, the coefficient of variation for depolarization times increased to 60–80%. Even a modest difference between the anesthetic groups might thus be easily missed. In fact, inspection of figure 3 suggests that depolarization times in the isoflurane animals were slightly longer than with halothane, at least at CBF_{LDF} values up to 15 units. However, the variability seen in humans with cerebrovascular disease is certainly much larger than in the narrowly defined circumstances reported here. In view of the difficulty demonstrating a difference in the experimental laboratory, it is unlikely that an important difference can be shown in humans. These factors in combination make it unlikely that isoflurane anesthesia will prove to be clinically protective in commonly encountered situations.

In summary, the results of this study support those presented in our earlier work.[12] Using LDF, we were unable to detect any anesthetic-related difference in the incidence of depolarization for the various hypotension groups or in the CBF threshold associated with ischemic cortical depolarization. In addition, whereas there was a clear relation between CBF_{LDF} and the delay until depolarization, there was no clear difference between anesthetics, except when CBF = 0. There did not appear to be any CBF "window" in which isoflurane resulted in dramatic prolongation of depolarization times. It is possible that this anesthetic might influence other electrical or biochemical events that might affect postischemic outcome and it is possible that significant prolongation of depolarization times might be found under other conditions (*e.g.*, higher isoflurane concentrations). However, if depolarization is a reasonable measure of the time to critical depletion of energy stores, then isoflurane–N_2O anesthesia as given here is unlikely to convey any energy-related protection relative to an equi-MAC concentration of halothane–N_2O.

References

1. Michenfelder JD, Theye RA: The effects of anesthesia and hypothermia on canine cerebral ATP and lactate during anoxia produced by decapitation. ANESTHESIOLOGY 33:430–439, 1970

2. Salford LG, Plum F, Brierley JB: Graded hypoxia-oligemia in rat brain: I. Biochemical alterations and their implications. Arch Neurol 29:227–233, 1973

3. Pulsinelli WA, Duffy TE: Regional energy balance in rat brain after transient forebrain ischemia. J Neurochem 40:1500–1503, 1983

4. Obrenovitch TP, Garofalo O, Harris RJ, Bordi L, Ono M, Momma F, Bachelard HS, Symon L: Brain tissue concentrations of ATP, phos-

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phocreatine, lactate, and tissue pH in relation to reduced cerebral blood flow following experimental acute middle cerebral artery occlusion. J Cereb Blood Flow Metab 8:866–874, 1988

5. Williams SR, Crockard HA, Gadian DG: Cerebral ischaemia studied by nuclear magnetic resonance spectroscopy. Cerebrovasc Brain Metab Rev 1:91–114, 1989

6. Astrup J, Rehncrona S, Siesjo BK: The increase in extracellular potassium concentration in the ischemic brain in relation to the preischemic functional activity and cerebral metabolic. Brain Res 199:161–174, 1980

7. Harris RJ, Symon L, Branstron NM, Bayhan M: Changes in extracellular calcium activity in cerebral ischemia. J Cereb Blood Flow Metab 1:203–209, 1981

8. Harris RJ, Symon L: Extracellular pH, potassium, and calcium activity in progressive ischaemia of rat cortex. J Cereb Blood Flow Metab 4:178–186, 1984

9. Hansen AJ: Effect of anoxia on ion distribution in the brain. Physiol Rev 65:101–148, 1985

10. Hansen AJ, Zeuthen T: Extracellular ion concentrations during spreading depression and ischemia in the rat brain cortex. Acta Physiol Scand 113:437–445, 1980

11. Hansen AJ, Nedergaard M: Brain ion homeostasis in cerebral ischemia. Neurochem Pathol 9:195–209, 1988

12. Verhaegen MJ, Todd MM, Warner DS: A comparison of cerebral ischemic flow thresholds during halothane/N_2O and isoflurane/N_2O anesthesia in rats. ANESTHESIOLOGY 76:743–754, 1992

13. White PF, Johnston PR, Eger EI: Determination of anesthetic requirements in rats. ANESTHESIOLOGY 40:52–57, 1974

14. Todd MM, Weeks JB, Warner DS: The failure of a focal cryogenic brain injury to alter halothane MAC in the rat. ANESTHESIOLOGY 79:139–143, 1993

15. Newberg LA, Milde JH, Michenfelder JD: The cerebral metabolic effects of isoflurane at and above concentrations that suppress cortical electrical activity. ANESTHESIOLOGY 59:23–28, 1983

16. Todd MM, Drummond JC: A comparison of the cerebrovascular and metabolic effects of halothane and isoflurane in the cat. ANESTHESIOLOGY 60:276–282, 1984

17. Newberg LA, Michenfelder JD: Cerebral protection by isoflurane during hypoxemia or ischemia. ANESTHESIOLOGY 59:29–35, 1983

18. Warner DS, Deshpande JK, Wielock T: The effect of isoflurane on neuronal necrosis following near-complete forebrain ischemia in the rat. ANESTHESIOLOGY 64:19–23, 1986

19. Nehls DG, Todd MM, Spetzler RF, Drummond JC, Thompson RA, Johnson R: A comparison of the cerebral protective effects of isoflurane and thiopental during temporary focal ischemia in primates. ANESTHESIOLOGY 66:453–464, 1987

20. Gelb AW, Boisvert DP, Tang C, Lam AM, Marchak BE, Dowman R, Mielke BW: Primate brain tolerance to temporary focal cerebral ischemia during isoflurane- or sodium nitroprusside-induced hypotension. ANESTHESIOLOGY 70:678–683, 1989

21. Warner DS, Zhou J, Ramani R, Todd MM: Reversible focal ischemia in the rat: Effects of halothane, isoflurane, and methohexital anesthesia. J Cereb Blood Flow Metab 11:794–802, 1991

22. Ruta TS, Drummond JC, Cole DJ: A comparison of the area of histochemical dysfunction after focal cerebral ischaemia during anaesthesia with isoflurane and halothane in the rat. Can J Anaesth 38: 129–135, 1991

23. Sano T, Drummond JC, Patel PM, Grafe MR, Watson JC, Cole DJ: A comparison of the cerebral protective effects of isoflurane and mild hypothermia in a model of incomplete forebrain ischemia in the rat. ANESTHESIOLOGY 76:221–228, 1992

24. Baughman VL, Hoffman WE, Miletich DJ, Albrecht RF, Thomas C: Neurologic outcome in rats following incomplete cerebral ischemia during halothane, isoflurane, or N_2O. ANESTHESIOLOGY 69:192–198, 1988

25. Milde LN, Milde JH, Lanier WL, Michenfelder JD, Gallagher W, Koenig R, Kroening A, Phelps L, Wilson R: Comparison of the effects of isoflurane and thiopental on neurologic outcome and neuropathology after temporary focal cerebral ischemia in primates. ANESTHESIOLOGY 69:905–913, 1988

26. Todd MM, Warner DS: A comfortable hypothesis reevaluated: Cerebral metabolic depression and brain protection. ANESTHESIOLOGY 76:161–164, 1992

27. Messick JM, Casement B, Sharbrough FW, Milde LN, Michenfelder JD, Sundt TM: Correlation of regional cerebral blood flow (rCBF) with EEG changes during isoflurane anesthesia for carotid endarterectomy: Critical CBF. ANESTHESIOLOGY 66:344–349, 1987

28. Blume WT, Ferguson GG, McNeill DK: Significance of EEG changes at carotid endarterectomy. Stroke 17:891–897, 1986

29. Michenfelder JD, Sundt TM, Fode N, Sharbrough FW: Isoflurane when compared with enflurane and halothane decreases the frequency of cerebral ischemia during carotid endarterectomy. ANESTHESIOLOGY 67:336–340, 1987

30. Stern MD, Lappe DL, Bowen PD, Chimosky JE, Holloway GA, Keiser HR, Bowman RL: Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. Am J Physiol 232:H441–H448, 1977

31. Skarphedinsson JO, Harding H, Thoren P: Repeated measurements of cerebral blood flow in rats: Comparisons between the hydrogen clearance method and laser Doppler flowmetry. Acta Physiol Scand 134:133–142, 1988

32. Eyre JA, Essex TJH, Flecknell PA, Bartholomew PH, Sinclair JI: A comparison of measurements of cerebral blood flow in the rabbit using laser Doppler spectroscopy and radionuclide labelled microspheres. Clin Phys Physiol Meas 9:65–74, 1988

33. Lindsberg PJ, O'Neill JT, Paakkari IA, Hallenbeck JM, Feuerstein G: Validation of laser-Doppler flowmetry in measurement of spinal cord blood flow. Am J Physiol 257:H674–H680, 1989

34. Dirnagl U, Kaplan B, Jacewicz M, Pulsinelli W: Continuous measurement of cerebral cortical blood flow by laser-Doppler flowmetry in a rat stroke model. J Cereb Blood Flow Metab 9:589–596, 1989

35. Haberl RL, Heizer ML, Marmarou A, Ellis EF: Laser-Doppler assessment of brain microcirculation: Effect of systemic alterations. Am J Physiol 256:H1247–1254, 1989

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