Local Cerebral Blood Flow and Glucose Utilization during Isoflurane Anesthesia in the Rat

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Volatile anesthetic agents have profound and heterogeneous effects on global and local cerebral blood flow (l-CBF) and metabolism. The relationship between l-CBF and local cerebral glucose uptake (l-CMRg) during isoflurane anesthesia is unknown. Because these relationships might influence neuronal homeostasis during periods of cerebral ischemia of different causes, it becomes important to understand them. Accordingly, the authors evaluated the l-CBF and l-CMRg effects of isoflurane with quantitative autoradiography in normal rats. As the dose of isoflurane increased in a stepwise fashion to 0.5, 1.0 (1.38%), 1.5, and 2.0 MAC levels, the number of structures with a significant (P < 0.05) l-CBF increase or l-CMRg decrease became greater. At each respective MAC level l-CBF was increased in 0%, 11%, 34%, and 30%, while l-CMRg decreased in 11%, 70%, 74%, and 81% of the structures in which autoradiographic measurements were performed. Between 1.5 MAC and 2.0 MAC the l-CMRg decrease stabilized at about 50% to 70% of cerebral metabolic values obtained in awake control rats in association with attainment of a burst-suppression pattern of electroencephalogram. In contrast to these general changes, l-CMRg in two subcortical limbic system structures (dente gyrus and interpeduncular nucleus) did not decrease, even at the highest doses of isoflurane. L-CBF was significantly (P < 0.05) increased only at the highest dose ranges (1.5–2.0 MAC) and increased from 34% to 238% in about one-third of the structures evaluated. Isoflurane anesthesia causes heterogeneous changes in l-CBF and metabolism, which are most apparent at doses of above 1.0 MAC. Differences in l-CBF/l-CMRg ratio patterns during isoflurane anesthesia suggest, at least in part, that cerebral flow and metabolic changes may proceed through unrelated regulatory mechanisms. (Key words: Anesthetics, volatile; isoflurane; MAC; Brain; blood flow, regional; glucose utilization; metabolism, regional.)

![Image](https://example.com/image.png)

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VOLATILE ANESTHETICS are known to cause dose-dependent global increases in cerebral blood flow (CBF) and to decrease overall cerebral metabolic rate (CMR) in humans and animals. However, the brain is structurally, functionally, and metabolically a heterogeneous organ, and distinct local cerebral blood flow (l-CBF) and metabolic effects occur during anesthesia. Isoflurane appears to possess unique cerebral hemodynamic and metabolic properties when compared with other volatile anesthetics; it depresses metabolism to a greater degree, increases CBF less, and does not appear to facilitate seizure discharges. These properties suggest that isoflurane may offer some degree of protection to hypoxic or ischemic brains. Possible utilization of isoflurane brain protection requires an understanding of isoflurane's influence on l-CBF–metabolism relationships during its administration to normal and ischemic brains. This information may help select clinical conditions appropriate for isoflurane application as well as improve our understanding of factors regulating CBF during anesthesia. In the present study, we examined the effects of isoflurane on l-CBF and local cerebral metabolic rate for glucose (l-CMRg) in normal rats.

Methods

L-CBF and metabolism were independently measured in 27 brain structures with 14C-iodoantipyrine (14C-IAP) and 14C-2-deoxyglucose (14C-2-DG) quantitative autoradiographic techniques. Sixty-one Sprague-Dawley female rats, weighing 283 ± 6 g (mean ± SD) were divided into groups for measurement of either l-CBF or l-CMRg at different dose levels of isoflurane.

The rats were briefly anesthetized with 2.0% isoflurane in 70% nitrogen and 28% oxygen for performance of a tracheostomy and femoral vessel cannulation of both arteries and veins. This vascular access was used for arterial blood pressure monitoring, venous administration of

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drugs, injection of isotope, and rapid, intermittent sampling of arterial blood for isotope counting via a continuously flowing, low dead space arteriovenous shunt. All incision sites were infiltrated with bupivacaine (less than 0.3 ml of 0.25% solution). Animals were paralyzed (pancuronium bromide 0.2 mg every 30 min) and mechanically ventilated. Heparin (200 IU) was administered to prevent catheter clotting.

ECG and bilateral frontoparietal EEG (gain of 50 µV/cm intermittently) were monitored. Rectal temperature was servocontrolled to 37.0°C with a heating lamp. Rats were then randomized into eight groups, depending on the concentration of isoflurane (0.5 MAC, 1.0 MAC = 1.38%, 1.5 MAC, and 2.0 MAC). Isoflurane was administered with a Drager® vaporizer, which was previously calibrated by gas chromatography. During the stabilization period (at least 60 min) at each isoflurane MAC level, the ventilator was adjusted to obtain the desired arterial blood gas composition, which was reconfirmed either just prior to or during the measurement of l-CBF or l-CMRgl. Blood for hematocrit determinations was drawn just prior to isotope infusions and immediately after decapitation. No significant (P > 0.05) hematocrit differences between the predecapitation and postdecapitation samples were found, and the results of each pair of determinations were averaged.

The conscious control groups were acclimated to a plexiglass restraining cage for at least 4 h/day over a 10-day period. The same restraining-cage method was maintained during either l-CBF or l-CMRgl determination. Anesthesia and surgical preparation were the same as for the isoflurane groups, except that tracheostomy was omitted. The EEG and ECG were not recorded, and the animals recovered from anesthesia in the cage for at least 2 h prior to either l-CBF or l-CMRgl measurement. During this period, they had free access to food and water, and breathed 30% oxygen in nitrogen given with a head hood.

**Quantitative Autoradiography**

At the end of the stabilization period, 1–2 ml of whole blood from a donor rat was given. Following this, 75 µC/kg of 14C-IAP (New England Nuclear, NEC-712®) dispensed in 1.0 ml of normal saline was infused at a constant rate for 30 s for l-CBF measurement. During the infusion period, 16–18 timed arterial samples (20 µl) were continuously collected for determination of arterial isotope activity by liquid scintillation counting (Nuclear Chicago, ISOCAP 300®).

Prior to l-CMRgl measurement, 1–2 ml of donor blood was given, and this was repeated 10–15 min after administration of the deoxycyglucose tracer. For l-CMRgl measurement, a bolus of 14C-2-DG (100 µC/kg; Amershansham) was given intravenously. Timed collection of 22 arterial blood samples (50–100 µl) over a 30 min period followed, and plasma glucose levels and arterial 14C-2-DG activity were determined.

At the end of either measurement period, the rat was decapitated and the brain was rapidly removed and frozen in 2-methyl-butane cooled to −35°C with Freon-XXII®. The brain was sectioned in a cryostat (−20°C) and the 20 µm sections were rapidly dried on a hot plate (60°C) and subsequently exposed, along with six 14C-methyl-methacrylate calibrated standards, to single-emulsion x-ray film (Kodak SB-5®) for 11 days. Following film development, optical densities were determined with an auto-scanning densitometer (Optronics, P-1000, International, Inc.) with an aperture of 200 µm, and all data were collected on-line with a Prime® computer for calculation of l-CBF and l-CMRgl according to equations developed by Reivich et al. (modified by Sakiura et al.) and Sokoloff et al. Ten–seven brain regions or structures were evaluated. For calculation of l-CMRgl, the lumped constant was 0.48.

**Statistical Analysis**

The data were analyzed by the one-way analysis of variance (ANOVA) for equality of mean values between groups with the Levine test employed to determine the application of separate or pooled t statistics for the ANOVA. Differences between specific groups were tested with the t test employing Bonferroni corrections for multiple comparisons.

**Results**

Physiologic conditions existing during the cerebral flow and metabolic measurements are summarized in table 1. There was a significant trend (greater in the l-CBF group) for mean arterial pressure to be lower at higher isoflurane levels. In both groups, pH also decreased slightly and plasma glucose was significantly increased from control at the 2.0 MAC dose. Otherwise, physiologic conditions between the flow and metabolism groups were comparable at the same isoflurane dose levels. The EEG showed progressive depression as the dose of isoflurane was increased. Occasional sharp waves appeared at and above 1.0 MAC of isoflurane. Figure 1 shows a representative, typical EEG during isoflurane anesthesia in rats.

Table 2 summarizes the l-CBF and l-CMRgl changes during administration of different levels of isoflurane anesthesia. When l-CMRgl changes are compared with the control state, there was a general trend for metabolism to decrease progressively (i.e., more structures involved and lower l-CMRgl) as the dose of isoflurane was increased. L-CBF remained statistically unchanged in all structures until it increased at the 1.5 MAC and 2.0 MAC...
levels of isoflurane. Over the entire isoflurane dose range, 
1-CMR$_q$ was never significantly increased in any structure; 
nor was l-CBF significantly decreased. However, l-CBF 
and l-CMR$_q$ changes were heterogeneous in the sense that 
significant changes in these variables were not necessarily 
coupled, and their distribution with regard to specific 
structures and degree of change shifted at different dose 
levels.

At 1.0, 1.5, and 2.0 MAC of isoflurane 70%, 74%, and 
81% of the structures, respectively, had significant l-CMR$_q$ 
decreases. Between 1.0 to 2.0 MAC some extrapyramidal 
and limbic subcortical structures were resistant to the 
l-CMR$_q$ depressive actions of isoflurane at the higher dose 
ranges. These included: 1) substantia nigra; 2) dentate gyri 
us (DG); 3) interpeduncular nucleus (IN); and 4) some 
white matter structures.

The l-CBF increasing effect of isoflurane peaked at 1.5 
MAC as 33% of the brain structures had significant ($P$
$< 0.05$) l-CBF increases when compared with the awake 
rats. Although l-CBF tended to increase further in most 
structures at 2.0 MAC, significant individual structure-
flow increases occurred less frequently than at 1.5 MAC 
due to a wider SD range at the higher isoflurane levels.

Figure 2 indicates the median and range of the l-CBF l-CMR$_q$ ratio values at each isoflurane dose for all structures. 
Because the maximal l-CMR$_q$ decrease plateaued 
between 1.0 and 2.0 MAC of isoflurane ($P > 0.05$) and 
the l-CBF continued to increase between 1.0 and 1.5 MAC 
($P < 0.05$) in eight structures, the large increase in the 
l-CBF/l-CMR$_q$ ratio in these areas was mainly due to 
increased flows. Figure 3 indicates the anatomic distribution 
of some of these ratios in a coronal brain section taken at 
the level of the thalamus at the 1.5 MAC isoflurane level.

The distribution of the percentage change in the 
l-CBF/l-CMR$_q$ ratio for all structures studied at every dose 
level is shown in figure 4. In 21 brain regions this ratio 
progressively increased as the level of isoflurane anesthesia 
was deepened. In six areas the percentage alteration in 
the ratio decreased from a previously higher level when 
the inspired isoflurane level was increased from 1.5 MAC 
to 2.0 MAC. In five of these structures the decrease in the 
l-CBF/l-CMR$_q$ ratio was to levels still approximating 
a 300% increase from control ranges, while in two limbic 
system areas (IN and DG) the ratio decreased to values 
approximating a 100% increase above control. In these 
two areas (IN and DG), the l-CBF/l-CMR$_q$ ratio increase 
at each dose level was considerably less than that exhibited 
by the rest of the brain structures surveyed at the 2.0 
MAC level of isoflurane.

**Discussion**

Isoflurane caused dose-related, heterogeneous alterations 
in l-CBF and metabolism in the rat. At multiple dose 
levels, and in many structures, the changes in l-CBF and
<table>
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<tr>
<th>Table 2. Local Cerebral Blood Flow and Metabolism during Isoflurane Anesthesia</th>
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<tr>
<td><strong>Abbreviation</strong></td>
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<td><strong>Auditory System</strong></td>
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<td><strong>Sensorymotor system</strong></td>
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<td>Cortex</td>
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<td><strong>Myelinated fiber tract</strong></td>
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<td>Corpus callosum</td>
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<td>Cerebral association areas</td>
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<td>Frontal cortex</td>
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<td>Reticular formation</td>
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Values are mean ± SD.

L-CBF = local cerebral blood flow (ml·100 g⁻¹·min⁻¹); L-CMRg = local cerebral metabolic rate for glucose (µmol·100 g⁻¹·min⁻¹).

Significantly different from control: *P < 0.05; †P = 0.01; ‡P < 0.001.
l-CMR$_g$ caused by isoflurane in our rats are qualitatively similar to those reported when overall CBF and metabolism are measured by methods affording little or no spatial resolution (i.e., increased flow and decreased metabolism).  This phenomenon has been termed “uncoupling” of flow and metabolism and is an attribute of volatile anesthetics.  Our study indicates that uncoupling does not occur uniformly throughout all brain regions and supports the hypothesis that volatile, anesthetic-related cerebral vasodilation and metabolic depression are not necessarily directly related phenomena.

**L-CMR$_g$**

Decreases in l-CMR$_g$ and EEG activity ran roughly parallel courses and developed a nadir plateau effect at the higher isoflurane levels (1.0–2.0 MAC). The EEG alterations in our rats are basically the same as those observed in humans anesthetized with isoflurane, except that the sharp waves found in the rats are not prevalent in patients.$^{16,17}$

While most of the surveyed structures manifested a reduction in l-CMR$_g$, some notably did not (table 2). This finding adds support to other studies of the local brain metabolic response to anesthetics suggesting a heterogeneous reaction pattern. Structures within the auditory, sensorimotor, visual, frontal association, and most of the extrapyramidal systems exhibited a dose-dependent l-CMR$_g$ reduction with isoflurane (table 2). When compared with halothane and enflurane effects on these structures in rats and primates, the metabolic decrease achieved with isoflurane is more potent and reaches a maximal effect when EEG isoelectricity occurs, as already demonstrated in canines.$^{5,6,18}$ Despite increasing doses of isoflurane, l-CMR$_g$ was not reduced in the DG of the hippocampal areas, IN, and substantia nigra (except at 0.5 MAC) (table 2). Resistance to anesthetic metabolic

**Fig. 2.** Ratio of local cerebral blood flow/local cerebral metabolic rate for glucose (l-CBF/l-CMR$_g$) for all structures studied ($n = 27$) during control and isoflurane anesthesia expressed as the range (low to high) for each anesthetic level. O = the median ranked value for each range.

**Fig. 3.** Anatomic distribution of l-CBF/l-CMR$_g$ ratios in the rat brain at 1.5 MAC isoflurane. Coronal section is at level of medial dorsal nucleus of the thalamus and situated 1.0 mm posterior to the bregma. Abbreviations for structures are noted in table 2.
depression in the hippocampus and interpeduncular nucleus during anesthesia with either intravenous or inhalational agents has been previously reported. At sub-MAC doses of halothane anesthesia in rats, I-CMR$_g$ in the hippocampus and substantia nigra was increased while the IN metabolism remained unchanged. In a prior study from our laboratory, 1 MAC of enflurane increased I-CMR$_g$ in the hippocampus and IN; but data on the substantia nigra were not collected. From this we conclude that while volatile anesthetics decrease I-CMR$_g$ in superficial cerebral cortex to a variable degree, they have an even more heterogeneous metabolic effect on subcortical metabolism.

At the present state of knowledge, incorporation of these local metabolic findings into a holistic concept of neurophysiologic function during anesthesia is not possible. Isolated neurophysiologic interpretations of I-CMR$_g$ data are notoriously unreliable as changes in I-CMR$_g$ may be directly due to the anesthetic agents and/or the result of alterations in inhibiting or excitatory influences that may be spread over diverse neuronal networks. However, the heterogeneous I-CMR$_g$ changes during anesthesia do represent modifications in local brain function. In some studies, supported by neurophysiologic data, maintenance of relatively high metabolic rates in some limbic system structures is thought to be due to disinhibition or direct stimulation and related to epileptogenicity demonstrated with ketamine, lidocaine, fentanyl, and enflurane. Although seizures did not occur in our isoflurane rats, sharp waves were recorded. Absence of seizures with isoflurane and halothane may be due to the relative resistance of the substantia nigra to anesthetic depression, as this region has been reported to possess anticonvulsant activity mediated by gamma-aminobutyric acid.

### L-CBF

Several factors render interpretation of the l-CBF changes more difficult than I-CMR$_g$ alterations. L-CMR$_g$ is relatively independent of minor alterations in the physiologic state (e.g., $P_{aCO_2}$ and arterial blood pressure), while CBF is exquisitely sensitive to changes in $P_{aCO_2}$ and arterial blood pressure when the CBF autoregulatory limits are exceeded. Also, vasodilation caused by volatile anesthetics further modifies autoregulation, rendering CBF even more dependent on arterial blood pressure. What cannot be clearly defined is the degree to which isoflurane and/or the CBF autoregulatory response to reduced blood pressure contribute to the cerebral vasodilation. Thus, minor differences in arterial blood pressure among animals in each MAC group could be expected to cause relatively large differences in l-CBF. Further complicating analysis of the l-CBF data are reports of local heterogeneity in L-CBF sensitivity to $P_{aCO_2}$ and in the autoregulatory response. Maintenance of higher arterial blood pressures with volume loading and/or vaspressors might increase the low l-CBF/I-CMR$_g$ ratios found in the DG and IN at 2.0 MAC of isoflurane.

### CBF–CMR COUPLING

At 0.5 MAC isoflurane, l-CMR$_g$ was significantly ($P < 0.05$) reduced in about 70% of the structures surveyed, while l-CBF remained statistically ($P < 0.05$) unchanged.
from control values in 100% of the structures (table 2). Actually, statistical analysis of our data does not reveal the fact that at 0.5 MAC of isoflurane I-CBF decreased from the control state in every instance. Thus, a downward trend in I-CBF (-8% to -38%) occurred in gray matter structures at 0.5 MAC at a time when I-CMR$_g$ declined between -30% to -51%. This may represent some initial maintenance of the flow–metabolic couple that exists in the awake state and during anesthesia with many of the intravenous anesthetic agents. Coupling of the I-CBF to I-CMR$_g$ changes may be then further modified by a direct vasodilator action of the volatile anesthetic at higher doses.

Furthermore, coupling of CBF to metabolism in order to maintain a set tissue or cerebral venous oxygen tension seems unlikely, as in most instances L-CBF increased significantly in the absence of any I-CMR$_g$ change at higher isoflurane dose levels. Based on the recognized, direct vascular smooth muscle relaxant action of volatile anesthetics, it may be speculated that uncoupling of flow from metabolism during isoflurane anesthesia is due to direct cerebral vascular dilation, as a process separate and distinct from the anesthetic's metabolic depressant effects and similar to that seen with nonanesthetic cerebral vasodilators.

Presently, the clinical significance of the heterogeneous L-CBF/I-CMR$_g$ uncoupling of isoflurane remains unknown. Uncoupling of the L-CBF/I-CMR$_g$ relationship may have detrimental effects during extremes of cerebral perfusion pressure. For instance, when local cerebral perfusion pressure is reduced secondary to cerebral vascular occlusion during deep anesthesia with halothane, isoflurane, thiopental, or pentobarbital, very different neurologic outcomes occur. The volatile agents appear to increase neurologic damage, while the barbiturates actually reduce it. In this situation, local flow and metabolic factors may be crucial determinants of the adequacy of residual or collateral flow. Modification of intrinsic L-CBF/I-CMR$_g$ regulatory mechanisms by volatile anesthetics may increase the ischemic insult. Similar pathogenetic circumstances may exist when generalized perfusion pressure reductions result in hippocampal dentate lesions, and our study found the lowest L-CBF/I-CMR$_g$ ratio in this region during high doses of isoflurane.

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References


