

## Effects of Isoflurane Anesthesia on Local Cerebral Glucose Utilization in the Rat

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The effect of isoflurane anesthesia on local rate of glucose utilization was investigated in the rat brain by means of the autoradiographic  $^{14}\text{C}$ -2-deoxyglucose method. Local cerebral glucose utilization (LCGU) was measured in 26 neuroanatomic structures of awake and isoflurane-anesthetized rats. Isoflurane anesthesia (1.5% inspired) caused both increases and decreases in LCGU. Significant reductions were found in all cortical areas examined and in primary sensory relay nuclei of central visual and auditory pathways. Among regions of the extrapyramidal motor system, LCGU was increased in substantia nigra pars compacta, and decreased in cerebellum, red nucleus, and ventral thalamus. Large increases in LCGU were observed in some structures of the limbic system such as the medial habenulo-interpeduncular system and the CA<sub>3</sub> field of hippocampus. LCGU was significantly reduced by isoflurane in the CA<sub>1</sub>-CA<sub>2</sub> field and dentate gyrus of hippocampus. These results are similar to previous findings on the LCGU response to other inhaled and intravenous anesthetics and further confirm the regional specificity of the effects of anesthetics on brain metabolism. (Key words: Anesthetics, volatile: isoflurane. Brain: glucose utilization; metabolism, regional. Metabolism: central nervous system.)

GENERAL ANESTHESIA IS associated with remodeling of the normal metabolic heterogeneity within the brain rather than uniform reduction of the cerebral metabolism. Studies of local cerebral glucose utilization (LCGU) have shown a considerable variability among different anesthetic agents. Whereas some anesthetics such as barbiturates<sup>1,2</sup> and chloral hydrate<sup>2,3</sup> reduce glucose utilization throughout the brain, other anesthetics such as ketamine<sup>4,5</sup> and halothane<sup>6,7</sup> produce both reductions and elevations in the metabolic rate of different brain regions.

LCGU, as determined by the quantitative autoradiographic  $^{14}\text{C}$ -2-deoxy-D-glucose ( $^{14}\text{C}$ -DG) method of Sokoloff *et al.*,<sup>1</sup> is coupled with functional activity in brain *in vivo* as well as in brain slices *in vitro*.<sup>8</sup> Therefore, this technique has been used to produce anatomic maps of functional changes associated with general anesthesia in an attempt to explain clinical properties of different anesthetics.

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The aim of the present study was to characterize the effects of the inhalational anesthetic isoflurane on rat brain glucose utilization. The concentration of the anesthetic used in this study was similar to the reported 1 MAC values for spontaneously breathing young rats.<sup>9,10</sup> Because isoflurane, unlike its isomer enflurane,<sup>11</sup> does not have convulsive properties,<sup>12</sup> we were particularly interested in comparing the metabolic changes observed during isoflurane anesthesia with those reported for enflurane.<sup>13</sup>

### Materials and Methods

Twelve male, 3-month-old Fischer-344 rats (Charles River Italia, Como, Italy) were used. Six served as control and six were treated with isoflurane (Forane®, Abbott, Latina, Italy). They were allowed free access to food and water until the morning of the experiment.

Animals were anesthetized with 2% isoflurane during a surgical procedure for the insertion of femoral arterial and venous polyethylene catheters (PE-50), that required approximately 15 min. Following surgery, the hindlimbs were restricted by means of a loosely applied plaster cast that allowed the rat to move its head and forequarters freely.

Control rats were allowed to recover from the effects of isoflurane for 4 h in a temperature-controlled, sound-insulated wooden box before iv injection of  $^{14}\text{C}$ -DG. Isoflurane-treated rats, spontaneously breathing, were placed in a plexiglass chamber of approximately 1 l volume. Isoflurane 1.5% was administered *via* a previously calibrated vaporizer (Fortec®, Cyprane, Keighley, UK) with compressed air as carrier gas ( $5\text{ l} \cdot \text{min}^{-1}$ ) with oxygen added to maintain  $\text{PaO}_2$  close to that of controls. Animals were maintained in a steady anesthetic state for 1 h prior to the iv injection of  $^{14}\text{C}$ -DG, and then for the entire experimental period.

Hematocrit, mean arterial pressure (MAP), heart rate, body temperature,  $\text{pH}_a$ ,  $\text{PaO}_2$ , and  $\text{PaCO}_2$  were monitored throughout the experiment. Body temperature of the rats was maintained normothermic by regulated external heating.

LCGU was determined after an iv bolus injection of  $125\text{ }\mu\text{Ci/kg}$  body weight of  $^{14}\text{C}$ -DG (Spec. Act.  $55\text{ }\mu\text{Ci/mmol}$ ; A.R.C., St. Louis, MO). Timed arterial blood samples were collected during the subsequent 50 min and centrifuged. Aliquots of plasma were taken for assessment of glucose (Glucose Analyzer II®, Beckman Instruments, Fullerton, CA) and of  $^{14}\text{C}$ -DG (Liquid Scintillation Spec-

trometer model B2450, Packard, Downers Grove, IL). At the end of the 50-min period, the animals were killed with an overdose of thiopental and decapitated, and the brains were rapidly removed and frozen in 2-methylbutane cooled to  $-50^{\circ}\text{C}$ .

Coronal sections ( $20\ \mu\text{m}$  thickness) were obtained with a cryostat (Cryocut E®, Reichert-Jung, West Germany) at  $-20^{\circ}\text{C}$ , and subsequently exposed, along with a set of previously calibrated  $^{14}\text{C}$ -methyl-methacrylate standards, to a single-emulsion x-ray Kodak SB-5® film for 7 days. Sections immediately adjacent to those used for autoradiography were stained with cresyl violet for histologic identification of brain structures and were used to compare autoradiograms with published anatomic sections of the rat brain.<sup>14,15</sup>

The autoradiograms were analyzed with a densitometer (MPM® 01K, Zeiss, West Germany). Six separate determinations of optical densities were made for each region in both left and right sides of the brain, and the means for the two sides were averaged.

LCGU was calculated from brain and plasma radioactivities and plasma glucose concentration, using equations and constants for transport and phosphorylation of  $^{14}\text{C}$ -DG given by Sokoloff *et al.*<sup>1</sup>

Data were analyzed for statistical significance by Student's *t* test for group analysis.  $P < 0.05$  was considered statistically significant.

## Results

Most of the physiologic parameters monitored were not significantly different in the anesthetized animals (table 1). Only MAP was significantly lower (21%) in the isoflurane-anesthetized rats; however, it remained within

TABLE 2. Effects of Isoflurane on Local Cerebral Glucose Utilization (LCGU) in Fischer-344 Rats

Brain Region	Control	Anesthetized	Difference (%)
LCGU ( $\mu\text{mol} \cdot 100\ \text{g}^{-1} \cdot \text{min}^{-1}$ )			
Cortical areas			
Prefrontal	$88 \pm 2$	$49 \pm 3$	$-44 \pm$
Motor	$84 \pm 6$	$65 \pm 4$	$-23^*$
Somatosensory	$93 \pm 3$	$41 \pm 1$	$-56 \pm$
Auditory	$116 \pm 5$	$55 \pm 3$	$-53 \pm$
Visual	$94 \pm 8$	$53 \pm 3$	$-44 \pm$
Sensory system			
Superior colliculus	$63 \pm 5$	$46 \pm 3$	$-27^*$
Inferior colliculus	$135 \pm 8$	$88 \pm 6$	$-35 \pm$
Medial geniculate	$84 \pm 6$	$35 \pm 2$	$-58 \pm$
Lateral geniculate	$62 \pm 3$	$50 \pm 2$	$-19 \pm$
Motor system			
Cerebellar cortex			
Cerebellar nuclei			
Substantia nigra	$51 \pm 6$	$33 \pm 3$	$-35^*$
compacta	$77 \pm 7$	$80 \pm 3$	$+4$
Red nucleus	$51 \pm 3$	$73 \pm 6$	$+43 \pm$
Caudate-putamen	$57 \pm 2$	$42 \pm 2$	$-26 \pm$
Globus pallidus	$72 \pm 3$	$73 \pm 6$	$+1$
Subthalamic nucleus	$41 \pm 1$	$36 \pm 5$	$-12$
Ventral thalamic nuclei	$71 \pm 2$	$67 \pm 5$	$-6$
	$72 \pm 3$	$35 \pm 2$	$-51 \pm$
Limbic system			
Anterior thalamic nucleus			
Mammillary bodies			
Medial habenula	$80 \pm 3$	$74 \pm 3$	$-7$
Lateral habenula	$86 \pm 5$	$85 \pm 6$	$-1$
Fasciculus	$64 \pm 6$	$119 \pm 9$	$+86 \pm$
retroflexus	$103 \pm 5$	$70 \pm 3$	$-32 \pm$
Interpeduncular nucleus	$64 \pm 5$	$140 \pm 14$	$+119 \pm$
	$89 \pm 7$	$117 \pm 7$	$+31^*$
Dorsal hippocampus			
CA <sub>1</sub> -CA <sub>2</sub> fields	$41 \pm 2$	$33 \pm 2$	$-20^*$
CA <sub>3</sub> field	$52 \pm 3$	$66 \pm 3$	$+27 \pm$
dentate gyrus	$45 \pm 2$	$32 \pm 2$	$-29 \pm$

Mean  $\pm$  SEM for six rats in each group.

Significantly different from control mean: \*  $P < 0.05$ ;  $\dagger P < 0.01$ ;  $\ddagger P < 0.001$ .

TABLE 1. Physiologic Parameters in Awake and Isoflurane-anesthetized Fischer-344 rats

	Control	Anesthetized
Hematocrit (%)	$49 \pm 2$	$50 \pm 1$
MAP (mmHg)	$125 \pm 8$	$99 \pm 4^*$
HR (beats/min)	$442 \pm 10$	$428 \pm 20$
Body temperature ( $^{\circ}\text{C}$ )	$37.3 \pm 0.4$	$37.1 \pm 0.2$
pH <sub>a</sub>	$7.40 \pm 0.02$	$7.38 \pm 0.02$
PaCO <sub>2</sub> (mmHg)	$38 \pm 0.5$	$43 \pm 1 \dagger$
PaO <sub>2</sub> (mmHg)	$115 \pm 3$	$120 \pm 5$
Plasma glucose (mg/dl)	$141 \pm 7$	$144 \pm 9$

MAP = mean arterial pressure; HR = heart rate.

Data represent mean  $\pm$  SEM for six animals in each group.

Significantly different from control mean: \*  $P < 0.05$ ;  $\dagger P < 0.01$ .

the autoregulatory range,<sup>16-18</sup> and therefore the decrease in MAP likely did not influence LCGU values. PaCO<sub>2</sub> increased by 13% in the anesthetized animals. This change is not large enough to alter substantially LCGU.<sup>19</sup>

LCGU was determined in 26 anatomically discrete structures of the brain (table 2). Representative brain autoradiograms from control and isoflurane-anesthetized rats are shown in figure 1.

Isoflurane significantly decreased LCGU 23–56% (average reduction 44%) in layer IV of all cortical regions examined. Significant declines in LCGU were also observed during isoflurane anesthesia in regions of central visual (superior colliculus and lateral geniculate) and auditory (inferior colliculus and medial geniculate) pathways.

In a group of brain regions connected with the extrapyramidal motor system, the metabolic effects of isoflurane were less homogeneous. In fact, isoflurane increased

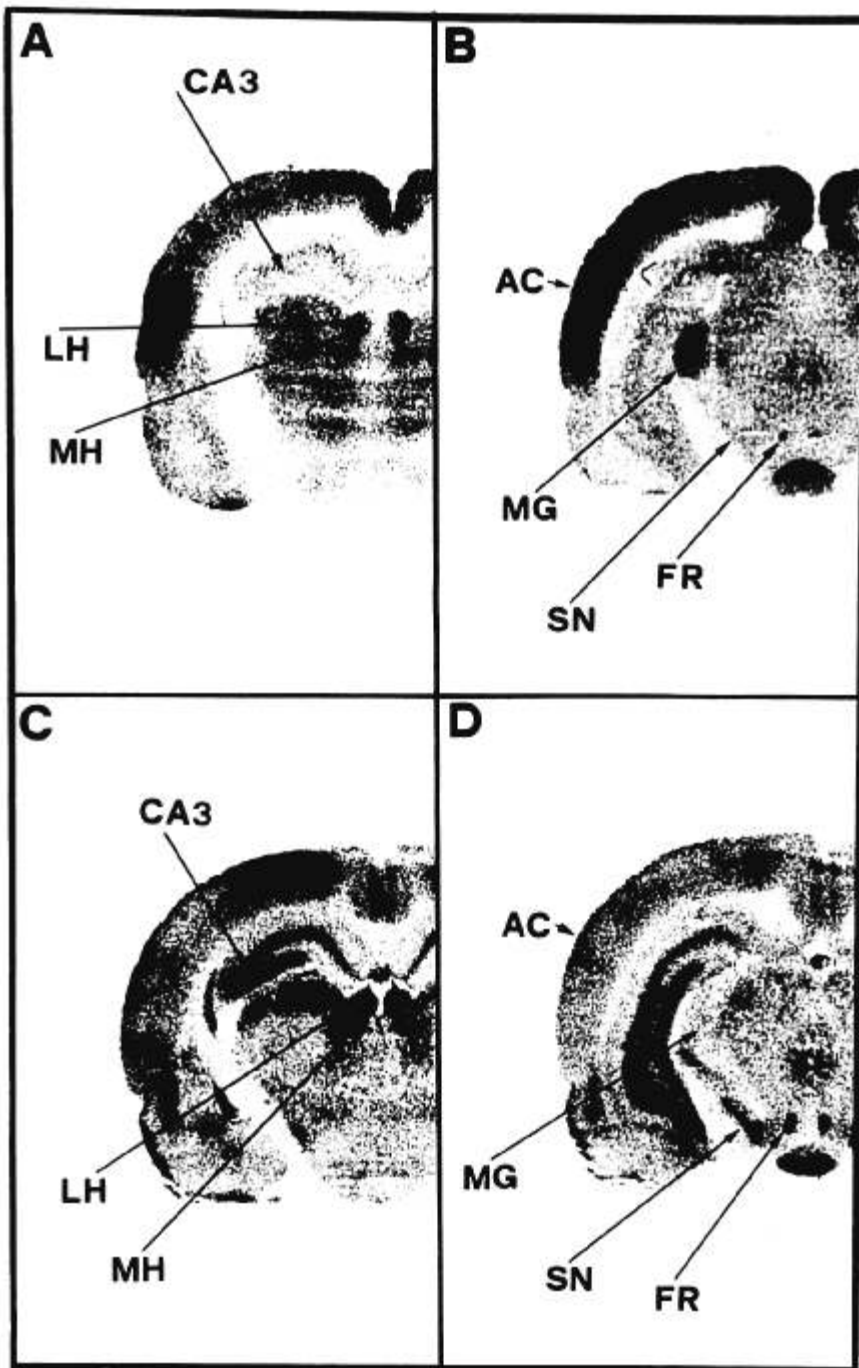


FIG. 1. Autoradiographs of  $^{14}\text{C}$ -DG uptake prepared from coronal rat brain sections at the levels of the habenular nuclei (A and C) and medial geniculate (B and D). Photomicrographs A and B show brain sections from control rats, C and D from isoflurane-anesthetized animals. Glucose utilization is proportional to relative optical densities. Isoflurane anesthesia increased grain densities in medial habenula (MH), CA<sub>3</sub> hippocampal field (CA<sub>3</sub>), substantia nigra pars compacta (SN), and fasciculus retroflexus (FR); it decreased  $^{14}\text{C}$ -DG uptake in lateral habenula (LH), medial geniculate (MG), and auditory cortex (AC) (original magnification  $\times 5$ ).

LCGU in substantia nigra pars compacta (+43%), whereas it significantly decreased metabolism in cerebellum (-35%), red nucleus (-26%), and ventral thalamic nuclei (-51%).

The greatest regional differences in LCGU values during isoflurane anesthesia were observed in areas of the limbic system. Isoflurane enhanced LCGU in the CA<sub>3</sub> field of the dorsal hippocampus (+27%), the medial ha-

benula (+86%), the interpeduncular nucleus (+31%), and the fasciculus retroflexus (+119%). On the other hand, significant reductions of LCGU (-20 to -32% compared with control) were found during isoflurane anesthesia in the lateral habenula, CA<sub>1</sub>-CA<sub>2</sub> fields, and dentate gyrus of hippocampus. LCGU was not affected by the anesthetic in the mammillary bodies and in the anterior thalamic nucleus.

## Discussion

LCGU has been determined during the anesthetic state in an attempt to elucidate mechanisms of general anesthesia or to correlate metabolic patterns with the different effects of various anesthetics. Widespread reductions of metabolism in gray matter structures have been observed during anesthesia produced by intravenous anesthetics (*e.g.*, barbiturates,<sup>1,2,20</sup> Althesin®,<sup>21,22</sup> ketamine,<sup>4,5</sup> and fentanyl<sup>23</sup>) and inhaled anesthetics (*e.g.*, ether,<sup>2</sup> N<sub>2</sub>O,<sup>20,24</sup> halothane,<sup>6,7</sup> and enflurane<sup>13</sup>). The greatest decreases were found in the cortex and regions of the somatosensory system, and were related to the sensory deprivation occurring during anesthesia.<sup>5</sup> This effect appears to be the only common feature of all anesthetics studied until now. Accordingly, in this study isoflurane significantly reduced LCGU in 15 of the 26 regions examined. Most of these regions were cortical areas and primary sensory relay nuclei. On the other hand, the medial habenulo-interpeduncular system, the CA<sub>3</sub> field of hippocampus, and the substantia nigra pars compacta displayed significant elevations in glucose utilization during isoflurane anesthesia. These findings are consistent with electrophysiologic studies showing that anesthetic agents cause both depression and excitation on different brain structures.<sup>25</sup>

A similar region-specific pattern of LCGU alterations was previously reported during halothane<sup>7</sup> and enflurane<sup>13</sup> anesthesia and was related to their clinical and neurophysiologic effects. For example, the metabolic activation of structures of the limbic system (such as hippocampus and the habenulo-interpeduncular system) may reflect the limbic seizure activity demonstrated during enflurane<sup>13</sup> and ketamine<sup>5</sup> anesthesia. Also, increases in the rate of glucose utilization in the same limbic regions under light anesthesia with halothane, which has no epileptogenic properties, have been thought to be related to the excitation observed during the first stage of anesthesia.<sup>7</sup> However, in the present study we observed a similar regional distribution of LCGU increases within limbic structures after administration of isoflurane, which does not have convulsive properties.<sup>12</sup> Furthermore, the inspired concentration of the anesthetic that has been used in our experiments (1.5%) is estimated to have produced, on the basis of duration of the experiments, an alveolar concentration of about 1.4%, which is very close to the reported 1 MAC value for young rats.<sup>9,10</sup> Therefore, these results do not reflect a subanesthetic state.

The increase of metabolism in the medial habenulo-interpeduncular system is a common effect of several intravenous and inhaled anesthetics,<sup>2,7,13,22</sup> regardless of their chemical structure. The functional significance of these findings is not clear. This pathway is not affected only by anesthetics because other centrally acting drugs

such as nicotine<sup>26</sup> similarly enhance LCGU in these structures. Moreover, lesion studies indicate that they do not represent a direct effect of anesthetics on these regions but rather an indirect, neurally mediated effect, which is abolished by destruction of afferent inputs.<sup>2,22</sup> Finally, the stimulatory effect of general anesthetics on LCGU of this pathway is dose-related, disappearing with increasing doses.<sup>13,27</sup> This finding suggests that increased metabolic activity in this system does not represent a stereotypic metabolic pattern of the anesthetic state but rather is sensitive to the level of anesthesia.

In conclusion, this study demonstrates that isoflurane produces heterogeneous changes in local brain metabolism, similar to those previously reported for halothane<sup>7</sup> and enflurane.<sup>13</sup> However, similar effects on LCGU may not exclude different properties. In particular, metabolic and neurophysiologic effects of general anesthetics should be compared cautiously because metabolic changes may not discriminate between regional excitatory or inhibitory events,<sup>28</sup> and may also represent indirect transynaptically mediated effects. We suggest that the regional nature of metabolic alterations may be better related to the depth of anesthesia at which LCGU was measured.

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