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Comparison of the Effects of Volatile Anesthetics on Brain Glucose Metabolism in Rats

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The objective of this investigation was to compare the effects of the commonly used volatile anesthetics on concentrations of plasma and cerebral glucose and cerebral intermediary metabolites. Fasted male Long-Evans rats were anesthetized with a volatile anesthetic and, after tracheostomy and paralysis, were mechanically ventilated. Each of three groups received one MAC concentration of anesthesia with halothane, enflurane, or isoflurane. At the end of 60–75 min of anesthesia, blood was sampled for arterial blood gas and plasma glucose analysis, and the brain was rapidly sampled and frozen for analysis of energy metabolites. Physiologic variables were maintained as follows: Pa_{CO_2} 30–40 mmHg, *p*Ha 7.20–7.40, Pa_{O_2} > 60 mmHg, MAP > 60 mmHg, and rectal temperature 37.5–38.5° C. Mean plasma glucose concentrations in the three groups were as follows (μMol/ml ± SEM): halothane, 7.45 ± .62; enflurane, 6.95 ± .22; isoflurane, 10.11 ± 1.00. Mean brain glucose concentrations in the three groups were (μMol/gm wet weight): halothane, 2.04 ± .20; enflurane, 2.07 ± .26; isoflurane, 3.04 ± .31. Plasma and brain glucose levels were significantly increased in the isoflurane group compared to the other two groups ($P < .05$) with no differences occurring in the brain/plasma glucose ratio among the three groups. No differences were present between groups in brain lactate, pyruvate, fructose diphosphate, malate, alpha-ketoglutarate, phosphocreatine, or adenine nucleotides. Thus, at one MAC concentration, major differences between volatile anesthetics on brain energy availability are not present, although isoflurane raised cerebral glucose levels. (Key words: Anesthetics, volatile: enflurane; halothane; Isoflurane. Brain: lactate; metabolism. Metabolism: glucose.)

ALTHOUGH THE EFFECTS of volatile anesthetics on cerebral metabolism have been the subject of several reports,[1-5] no single study has compared the cerebral metabolic effects of halothane, enflurane, and isoflurane in an animal preparation in which the clinical surgical situation is approximated. The purpose of this investigation was, under such conditions, to compare the effects of the commonly administered volatile anesthetics on concentrations of plasma and cerebral glucose and cerebral intermediary metabolites.

Methods and Materials

Male Long-Evans rats, 200–300 gm, obtained from Charles River Laboratory, were fasted overnight with free access to water.

The rats were divided into three groups based upon which anesthetic they received: halothane, enflurane, or isoflurane. For humane reasons, a comparable control group of paralyzed, ventilated, unanesthetized rats was not included in the experimental design. Institutional approval for these experiments was obtained. In each rat, anesthesia was induced with the anesthetic which was to be studied: 4% halothane, 7% enflurane, or 4% isoflurane for 3–5 min. Subsequently, tracheostomy was performed followed by intramuscular administration of d-tubocurarine, 0.45 mg, and insertion of biparietal electroencephalogram (EEG) leads and a rectal temperature probe. Mechanical ventilation was subsequently maintained for 60–75 min with a Harvard Rodent Respirator during which time anesthesia was maintained at one of the following inspired anesthetic concentrations delivered *via* vaporizor (calibrated with a Perkin-Elmer mass spectrometer): halothane 1.4%, enflurane 2.6%, or isoflurane 1.5% in 40% oxygen in nitrogen. These halogenated anesthetic concentrations were chosen to provide end-tidal concentrations approximately equivalent to MAC for rats after 1 h of anesthesia.[6,7] During this period, in addition, a left femoral polyethylene (PE50) arterial catheter was inserted *via* surgical cutdown, with local application with a cotton-tipped applicator of 1% lidocaine. Arterial blood pressure, EEG, and rectal temperature were recorded with a Gilson ICM-8H Polygraph. Experiments were performed between 8:00 AM and 5:00 PM, with there being no differences between the groups in the time of day that experiments were performed.

Forty-five minutes after induction of anesthesia Pa_{O_2}, Pa_{CO_2}, and *p*Ha were determined (BMS 3 MK2 Blood Gas Analyzer, Radiometer, Copenhagen), and the ventilator subsequently adjusted to produce a Pa_{CO_2} of 30–40 mmHg at the time of brain removal. Rectal temperature was maintained at 37.5–38.5° C with a heating lamp. Mean arterial pressure, *p*Ha, and Pa_{O_2} were not otherwise pharmacologically or physiologically manipulated (*i.e.*, pressors, bicarbonate, and positive end expiratory pressure were not used). Removed blood was re-

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placed with approximately equivalent volumes of normal saline. At the end of the experimental period, 1 ml of blood was withdrawn for subsequent arterial blood gas and plasma glucose determination after which the brain was rapidly sampled.

Brain Sampling

The apparatus used was the "freeze-blower" (Precision Medical Instruments, Shalimar, FL) developed by Veech *et al.*[2,4,8,9] Briefly, this device operates by simultaneously thrusting hollow, sharp stainless steel cannulae into either side of the calvarium, as compressed gas coming from one cannula hydraulically displaces forebrain out the opposing cannula onto a liquid-nitrogen precooled plate. Using this apparatus, it is possible to remove and freeze 0.5–1.0 gm forebrain samples in a fraction of a second, thereby minimizing sampling artifact.

Preparation of Brain and Blood Extracts

After rapid removal and freezing, brain tissue was stored at −70° C and, subsequently, was prepared using standard biochemical techniques.[8,10] Briefly, the brain was ground to a powder under liquid nitrogen, and was deproteinized with a methanol/concentrated HCL solution (495:5v/v) at −20° C followed by 1.2 N perchloric acid and 5 mM EGTA at 4° C. After centrifugation, the supernatant was neutralized and assayed for metabolites.

Plasma was prepared by addition of 0.5 M perchloric acid followed by cold centrifugation and neutralization, and storage at −70° C.

Analytical Methods

Brain and blood extracts were assayed using standard spectrophotometric techniques (Zeiss Instruments) utilizing enzymatic coupling to pyridine nucleotides.[11] With these techniques and the sample dilutions used in these experiments, changes of 0.003–0.024 μMol/gm of tissue can be detected.

Statistical Analysis

Data were analyzed by analysis of variance. Where this indicated statistically significant differences, Student-Newman-Keuls testing was performed (SAS Institute, Cary, NC).

Results

Physiologic data are summarized in table 1. There were no statistically significant differences between any of the groups in any of these physiologic variables. Biochemical data are summarized in table 2. Brain and plasma glucose were both higher in the isoflurane group ($P < .05$). Brain to plasma glucose ratio was 0.28–0.30 in the three groups, with no significant differences occurring between groups. Between the three anesthetics there were no significant differences in glucose-6-phosphate, fructose diphosphate, pyruvate, lactate, alpha-ketoglutarate, malate, phosphocreatine, ATP, ADP, or AMP.

TABLE 1. Physiologic Variables*

	Halothane	Enflurane	Isoflurane
MAP (mmHg)	79 ± 6	79 ± 9	91 ± 10
Pa_{O_2} (mmHg)	149 ± 14	190 ± 42	198 ± 31
Pa_{CO_2} (mmHg)	39 ± 0.4	38 ± 0.6	35 ± 1.4
*p*Ha	7.26 ± 0.01	7.31 ± 0.02	7.26 ± 0.01
T_R (°C)	38.0 ± 0.1	37.9 ± 0.1	38.0 ± 0.2
Weight (gm)	262 ± 13	258 ± 7	256 ± 5

MAP = mean arterial pressure; T_R = rectal temperature.
* Rats received the indicated anesthetics. See methods and materials for details of the experimental preparation. Values shown are the means ± SE of five rats.

TABLE 2. Brain Energy Metabolites*

	Halothane	Enflurane	Isoflurane
Glucose	2.04† (0.20)	2.07† (0.26)	3.04 (0.31)
Glucose-6-phosphate	0.13 (0.01)	0.14 (0.01)	0.16 (0.004)
Fructose diphosphate	0.05 (0.01)	0.05 (0.01)	0.06 (0.005)
Pyruvate	0.08 (0.01)	0.08 (0.004)	0.09 (0.01)
Lactate	0.49 (0.13)	0.63 (0.08)	0.73 (0.07)
α-ketoglutarate	0.10 (0.03)	0.15 (0.02)	0.10 (0.02)
Malate	0.27 (0.04)	0.17 (0.07)	0.21 (0.10)
Phosphocreatine	3.48 (0.32)	3.65 (0.12)	3.78 (0.17)
ATP	2.38 (0.19)	2.47 (0.12)	2.75 (0.09)
ADP	0.33 (0.03)	0.35 (0.02)	0.37 (0.01)
AMP	0.02 (0.004)	0.02 (0.005)	0.01 (0.002)
Plasma glucose	7.45† (0.62)	6.95† (0.22)	10.11 (1.00)
Brain/plasma glucose ratio	0.28 (0.03)	0.29 (0.03)	0.30 (0.02)

* Rats received the indicated anesthetics in 1 MAC concentrations. See methods and materials for details of the experimental preparation. Values shown are means (with the SE in parentheses) of five rats expressed as μMol/gm wet weight (μMol/ml for plasma glucose).
† Statistical significance compared with the isoflurane group ($P < .05$).

TABLE 3. Investigations of Metabolic Effects of Volatile Anesthesia

Reference	Species (Nutrition)	Surg/Physiol Prep*	Anes Tested (n)	Duration of Anes	Br Freezing Method†	Brain Glucose (μMol/gm wet wt)
Brunner, Passoneau, Molstad[1] 1971	Swiss-Webster mouse (fed)	No/spont vent	None (4–6) Hal 0.8% (9) Enf 1.1% (12)	? 1–4 h 1–6 h	Decap into freon or body into freon	1.40–1.66 1.75–2.52 2.99–3.65
Biebuyck, Hawkins[2] 1972	Rat (fed)	No/spont vent	None (6–8)	—	Rapid brain sampler	1.66
		No/spont vent	Hal 1.5% (6–8)	10–40 min	Rapid brain sampler	3.47
Nilsson, Busto[12] 1973	Wistar rat (?)	Yes/mech vent-A line	None (8) N_2O (10)	30 min ?30–60 min	Ponten funnel Ponten funnel	4.97‡ 3.56
Nilsson, Siesjo[3] 1974	Wistar rat (fed)	Yes/mech vent-A line	Fent sedation (6) N_2O (6) Hal 1% (6)	30 min 30 min 30 min	Ponten funnel Ponten funnel Ponten funnel	5.87‡ 5.30 4.18
Dedrick, Scherer, Biebuyck[4] 1975	Wistar rat (fed)	No/spont vent	None (11–22)	—	Rapid brain sampler	1.20
		Yes/mech vent-A line	Hal 1–1.5% (16)	60 min	Rapid brain sampler	2.66
Berntman, Carlsson, Hagerdal, Siesjo[14] 1976	Wistar rat (fed)	Yes/mech vent-A line	None (6) N_2O (6)	15 min >30 min	Ponten funnel Ponten funnel	4.42‡ 3.80
Ratcheson, Bilezikjin, Ferrendelli[17] 1977	Mouse (fed)	No/spont vent	None (11) N_2O (11)	— 30 min	Body into liq N_2 Body into liq N_2	1.30 1.65
McCandless, Wiggins[5] 1981	Swiss-Albino mouse (fasted)	No/spont vent	None (3–5) Hal 1% (3–5)	5 min 5 min	Body into liq N_2 Body into liq N_2	3.1§ 5.0§

A-line = arterial line; Anes = anesthesia; Br = brain; bx = biopsy; ctl = controls; ctx = cortex; Decap = decapitate; Enf = enflurane; Fent = fentanyl; froz = frozen; h = hour; Hal = halothane; Iso = isoflurane; m = minute; mech = mechanical; n = number of animals tested; physiol = physiological; prep = preparation; spont = spontaneous; surg = surgery; vent = ventilation.

* If a surgical procedure was required as part of the monitoring or experimental protocol, "yes" appears; if not, "no" appears. Use of mechanical *versus* spontaneous ventilation or intraarterial cannulation are so noted.

† "Rapid brain sampler" is the device described in this report. "Ponten funnel" is a funnel applied to the cortex of the brain into which liquid nitrogen is poured. "Decap into freon" refers to decapitation of an animal, allowing the head to drop into Freon or liquid nitrogen. "Body into liq N_2" refers to sacrificing an animal by inserting the entire body into liquid nitrogen.

‡ Stressed controls.

§ MicroMol/gm dry weight.

Discussion

The higher plasma glucose we observed with isoflurane in rats has also been observed in humans anesthetized with isoflurane.[12] This increase most likely contributed significantly to the higher brain glucose obtained with isoflurane in our experiments, as the brain/plasma glucose ratio was constant with all the halogenated anesthetics. Lower glucose utilization or increased blood brain barrier transport with isoflurane could also have accounted for it, but cannot be addressed specifically with our data.

The effects of inhaled anesthetics on brain biochemistry have been reported in a number of investigations.[1–5,13–17] However, variable anesthetic concentrations, animal models, types of control groups, physiologic control, nutritional state, and methods of sampling and freezing the brain have been used, making comparisons between different anesthetic agents difficult (table 3). This investigation addressed these problems by modeling the common clinical situation, in that a one MAC concentration of each halogenated anesthetic was administered during a surgical procedure with controlled ventilation following an overnight fast.

Initial review of the brain glucose concentrations determined in the investigations in table 3 suggests disagreement as to whether anesthesia increases or decreases brain glucose. However, when examined with respect to the nature of the control groups, the studies all appear to be consistent with each other and with our data. That is, all studies in which data were compared with stressed controls (*i.e.*, paralyzed and ventilated awake or lightly sedated)[3,12,14] conclude that volatile anesthetics decrease brain glucose, whereas comparison with minimally stressed controls demonstrates increased brain glucose

with volatile anesthetics.[1,2,5,17] Compared with minimally stressed unanesthetized controls from prior studies with similar methods in which brain glucose was 1 μMol/gm wet weight,[8-10] our data further suggest (without statistical comparison) that brain glucose is increased with volatile anesthesia.

Regardless of the control group, these studies all demonstrate no major effects of or differences between volatile anesthetics on cerebral concentrations of high energy phosphates or other intermediary metabolites.[1-5,13-17] Our data are consistent with these observations, as the levels of phosphocreatine and adenine nucleotides we observed are similar to those reported in unanesthetized rats.[8-10] We have, however, additionally demonstrated a relatively small increase in brain and plasma glucose with one MAC isoflurane anesthesia relative to one MAC halothane and enflurane anesthesia. Any deleterious contribution of this glucose increase to final neurologic outcome if cerebral ischemia occurs[18-20] may be a consideration in choosing an anesthetic if cerebral ischemia is likely to occur during an anticipated operative procedure.

In summary, we have demonstrated maintenance of adequate cerebral high energy phosphate content with administration of one MAC concentrations of halothane, enflurane, and isoflurane in rats. The primary difference between these anesthetics appears to be in plasma and brain glucose levels, with the highest concentration occurring with isoflurane.

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