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Comparison of Methohexital and Isoflurane on Neurologic Outcome and Histopathology Following Incomplete Ischemia in Rats

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Using a rat model of incomplete cerebral ischemia the effects of isoflurane (iso) and methohexital (metho) were compared with those of 70% nitrous oxide controls (N_2O). Two levels of incomplete cerebral ischemia were produced by right carotid occlusion plus hypotension for 30 min: moderate = 30 mmHg, $F_{I_{O_2}}$ = 0.30; severe = 25 mmHg, $F_{I_{O_2}}$ = 0.20. The iso doses (1 and 2 MAC) and metho doses (0.01 and 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$) were tested at each ischemic level. These iso and metho doses were selected because without ischemia they produced similar decreases in cerebral oxygen consumption (CMR_{O_2}) compared with that produced in N_2O controls. In the absence of ischemia, the electroencephalogram (EEG) was suppressed by 0.01 $mg \cdot kg^{-1} \cdot min^{-1}$ metho and 1 MAC iso and showed burst–suppression with 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ metho and 2 MAC iso. The EEG was further depressed by ischemia under all anesthetic conditions. Neurologic outcome was evaluated for 3 days following incomplete cerebral ischemia by using a graded deficit score (0 = normal, 5 = death associated with stroke). Following moderate ischemia all four anesthetic treatments improved outcome compared with N_2O controls, but after severe ischemia only 2 MAC iso significantly improved outcome. Neurohistopathology was evaluated on a scale of 0 to 40, 24 h after ischemia. The neurohistopathology score was significantly improved by all four anesthetic treatments compared with N_2O following moderate ischemia and was better with 2 MAC iso compared with 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ metho after both moderate and severe ischemia. These results show that both iso and metho improve outcome from cerebral ischemia compared with that associated with N_2O, but only 2 MAC iso resulted in an improved outcome following severe ischemia. This difference in outcome between the two anesthetics may be related to greater neuronal depression with iso, which may occur with little difference in cerebral metabolic depression. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, intravenous: methohexital. Anesthetics, volatile: isoflurane. Brain: blood flow; ischemia; metabolism; protection. Glucose. Measurement technique: EEG.)

FOR EXPERIMENTAL AND CLINICAL PURPOSES brain ischemia can be separated into two distinct types: complete ischemia in which cerebral blood flow (CBF) is stopped completely for a period of time and incomplete ischemia in which CBF is still present but is inadequate to prevent neuronal damage. This classification is important because several drugs have been shown to attenuate brain injury produced by incomplete ischemia (but not complete ischemia) based on their ability to decrease cerebral metabolic rate.[1–3] Isoflurane and barbiturates are considered to be effective in protecting the brain from incomplete ischemia because they both can silence neuronal electrical activity and decrease cerebral oxygen consumption (CMR_{O_2}) by approximately 50%.[4–8] If barbiturates and isoflurane both have the ability to depress CMR_{O_2} until EEG silence, the question arose as to whether one drug was superior in affording brain protection during incomplete ischemia. Nehls *et al.*[9] compared the protective effect of isoflurane with that of thiopental in the baboon with ischemia produced by middle cerebral artery occlusion (MCAO). They found that thiopental provided better neurologic outcome and less cerebral infarction than did isoflurane in doses producing maximum brain depression as indicated by EEG. Conversely, Milde *et al.*[10] reported that isoflurane and thiopental produced similar neurologic outcome and brain infarct size following MCAO in monkeys. These conflicting results question whether isoflurane or barbiturates improve outcome from ischemia and whether either anesthetic is better. To investigate this question, we compared the cerebral protective effects of iso and methohexital in doses that produced similar cerebral metabolic depression in a model of incomplete ischemia in rats. Methohexital was chosen as a short-acting barbiturate that allows similar recovery time from anesthesia as that from isoflurane. Our results show that both drugs protect the brain from incomplete ischemia but that 2 MAC isoflurane is more protective than N_2O and methohexital during severe ischemia.

Methods

These experiments were performed after approval from the Institutional Animal Care Committee.

EXPERIMENT 1. CEREBRAL BLOOD FLOW AND METABOLISM

To establish equipotent doses of methohexital and isoflurane, CBF and CMR_{O_2} were measured in rats during

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70% N_2O alone, and during both moderate and deep anesthesia with these two drugs without ischemia (n = 4–5 per group) (table 1). Following induction of anesthesia in a bell jar with isoflurane and orotracheal intubation, male Sprague-Dawley rats (350–450 g) were anesthetized with 1.7% inspired isoflurane. Both femoral arteries and one femoral vein were catheterized and the animal was paralyzed with vecuronium. In addition, the left ventricle was catheterized *via* the right subclavian artery, using pressure pulses to monitor proper catheter placement. This method of catheter placement allows brain perfusion by both carotid arteries with microsphere injections during the control period.[11] The skull was then exposed, a small hole was drilled over the posterior sagittal sinus, and a catheter was inserted to obtain sagittal sinus blood samples. At the completion of surgery the isoflurane was removed from the inspired gases and replaced with 70% N_2O. Following a 30-min stabilization period the first CBF measurement was made. Then either 0.01 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital or 1.4% inspired isoflurane concentration was started and 70% N_2 substituted for 70% N_2O. After an additional 30-min equilibration period a second CBF measurement was made. Following this test the methohexital infusion rate was increased to 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ and inspired isoflurane concentration increased to 2.8% and a third CBF measurement made after a 30-min equilibration period.

TABLE 1. Experimental Protocol

Experiment	Ischemia	Test Group (n)
1 CBF/CMR_{O_2}*	None	70% N_2O → low metho → high metho (4) 70% N_2O → 1 MAC iso → 2 MAC iso (4)
2 EEG†	Moderate → severe	70% N_2O (4), low metho (4), high metho (4), 1 MAC iso (4), 2 MAC iso (4)
3 Neurologic outcome‡	Moderate	70% N_2O (9), low metho (8), high metho (10), 1 MAC iso (10), 2 MAC iso (10)
	Severe	80% N_2O (9), low metho (8), high metho (8), 1 MAC iso (10), 2 MAC iso (12)
4 Histopathology§	Moderate	70% N_2O (4), low metho (4), high metho (5), 1 MAC iso (4), 2 MAC iso (6)

Low metho = 0.01 $mg \cdot kg^{-1} \cdot min^{1}$; high metho = 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$; 1 MAC iso = 1.47% inspired; 2 MAC iso = 2.8% inspired; mod ischemic = BP 30 mmHg, FI_{O_2} = 0.30; severe ischemia = BP 25 mmHg, FI_{O_2} = 0.20.

* CBF/CMR_{O_2} was measured three times in each rat: during N_2O, followed by low dose and then high dose metho, or N_2O followed by 1 MAC and then 2 MAC iso.

† EEG was tested under each baseline anesthetic treatment followed by moderate and then severe ischemia.

‡ Neurologic outcome was evaluated for 3 days following either moderate or severe ischemia with each drug treatment.

§ Histopathology was evaluated 24 h following moderate ischemia with each drug treatment.

Fifteen micron microspheres labeled with cobalt-57, tin-113, and scandium-146 (New England Nuclear, Boston, Massachusetts) were used. Previous studies have shown that repeated microsphere injections do not alter CBF measurements.[12] Stock solutions containing 500,000 microspheres/ml were suspended in isotonic saline with 0.01% Tween-80. Microspheres were vortexed for 1 min, 0.2 ml withdrawn (100,000 microspheres), injected into the left ventricle *via* the subclavian catheter (dead space = 0.06 ml), and flushed in with 0.2 ml saline over 20 s. Starting immediately before each microsphere injection and continuing 45 s after the end of each injection, blood was withdrawn from a femoral artery at 0.4 ml/min. This catheter was flushed between injections to ensure that contamination did not occur from previous injections. Mean arterial blood pressure was measured continuously to ensure blood pressure did not change appreciably during the microsphere injection. Each rat was killed, the brain removed and sectioned into right and left cortical and subcortical samples, and weighed. Cortical tissue typically contains a small percentage (less than 10%) of white matter. The activity of each microsphere in brain and blood samples was analyzed using a Nuclear Data 600 multichannel analyzer. CBF was analyzed according to the methods of Heymann *et al.*[13] using the following formula: CBF = (tissue activity/blood activity) × (withdrawal rate/tissue weight) × 100.

Arterial and sagittal sinus blood samples were taken following each microsphere measurement for blood gas, *p*H, and oxygen content determinations. Blood gas tensions were measured with an IL 1303 blood gas analyzer. Arterial–sagittal sinus O_2 content was calculated from measurements made with an IL 280 CO-oximeter and oxygen dissolved in the plasma. Cerebral cortex oxygen consumption (CMR_{O_2}) was calculated in each rat by multiplying cortex CBF times arterial–sagittal sinus O_2 content. Sagittal sinus blood derives mainly from cerebral cortical tissue with a small contamination from noncortical structures.[14]

Cerebral ischemia model. A similar method for producing cerebral ischemia was performed for experiments 2–4 (table 1). Nonfasted male Sprague-Dawley rats weighing 350–450 g were anesthetized with 1.7% isoflurane inspired concentration for surgical preparation. Following orotracheal intubation using a PE190 endotracheal tube, catheters were inserted into the right femoral artery and vein for continuous blood pressure recording and drug administration and into the right subclavian vein for blood withdrawal. The right common carotid artery was isolated and a loose ligature placed around it for later clamping

with a vascular clamp. Rats were paralyzed with vecuronium. Inspired isoflurane was then adjusted to 1.4% or 2.8% or was discontinued and replaced with 70% or 80% N_2O in oxygen (control rats) or an iv infusion of methohexital (0.01 or 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$). [Inspired isoflurane concentrations used here (1.4% and 2.8%) only approximate 1 and 2 MAC anesthesia because it has been shown that 30 min after administration, the isoflurane inspired to alveolar concentration ratio is 1.26.[15,16]] The lungs of isoflurane and methohexital treated rats were ventiated with 70% or 80% N_2 in O_2. Following a 30-min equilibration period, cerebral ischemia was produced by the combination of carotid occlusion using a Serrefine small vessel clamp and hemorrhagic hypotension to a mean blood pressure of 30 mmHg with $FI_{O_2} = 0.30$ (moderate ischemia) or 25 mmHg with $FI_{O_2} = 0.20$ (severe ischemia) for 30 min. Blood was withdrawn from the subclavian vein into a heparinized syringe, and a range of 2 mmHg was allowed for each hypotensive level. Arterial blood samples were obtained at the end of the equilibration period, during ischemia, and after 30 min of recovery from ischemia to measure plasma glucose, blood gas tensions, and *p*H. Plasma glucose was measured using a Yellow Springs glucose analyzer. Rectal temperature was maintained at 37° C using a Yellow Springs temperature servocontrolled thermistor, connected to an overhead heat lamp. Pa_{CO_2} was kept between 35 and 45 mmHg by adjusting ventilation throughout the study including recovery to extubation. Vecuronium was injected 0.1 mg/kg and infused at a rate of 0.04 $mg \cdot kg^{-1} \cdot min^{-1}$ to maintain paralysis. Arterial *p*H was maintained at normal levels with an 8.4% sodium bicarbonate infusion. The bicarbonate infusion rate approximated 0.05 ml/min in N_2O ventilated rats and 0.02 ml/min in the other anesthetic groups. At the end of the ischemic period the carotid artery was unclamped and the withdrawn blood slowly reinfused into the subclavian vein over 15 min. Withdrawn blood was heparinized but not warmed before reinfusion. Body temperature was maintained at 37° C during recovery. Following the 30-min recovery period the catheters were removed, the incisions closed following infiltration with bupivicaine. All rats received the same dose (0.5 mg). Rats were extubated when breathing spontaneously without the use of neostigmine. For N_2O- and isoflurane-ventilated rats, vecuronium and anesthetic treatments were continued until completion of 30 min of recovery period following ischemia. Extubation then occurred within the next 15 min. To produce approximately the same recovery time in high-dose methohexital-treated rats, the anesthetic and vecuronium infusion were stopped at the end of ischemia, whereas in low-dose methohexital-treated rats they were continued for 15 min of the recovery period. Following extubation all animals were maintained in single cages in a temperature and light-controlled environment breathing room air.

Experiment 2. Electroencephalography

The electroencephalogram (EEG) was evaluated for each drug treatment during both moderate and severe ischemia (n = 4 per treatment group). These rats were not evaluated for outcome from ischemia. The rats were prepared for the ischemia as described above. Additionally, the skull was exposed and screw electrodes inserted above the right cortex for bipolar EEG recordings. EEG was recorded from the ischemic hemisphere *via* a Grass bioelectric preamplifier connected to a Hewlett-Packard EEG amplifier. For each experimental group (N_2O controls, low-dose, and high-dose methohexital and isoflurane) the baseline EEG was recorded after a 30-min equilibration period. Cerebral ischemia was then produced by occluding the right carotid artery and decreasing the blood pressure to 30 mmHg with $FI_{O_2} = 0.30$ (moderate ischemia). The EEG was recorded during this 15-min test period. The blood pressure was then decreased to 25 mmHg and the FI_{O_2} decreased to 0.20 (severe ischemia). EEG was then recorded for an additional 15-min test period. The carotid occlusion was then released, blood slowly reinfused into the subclavian vein, and the recovery EEG recorded for 30 min. EEG was intermittently recorded from the nonischemic (left) hemisphere in selected animals, but these EEG changes consistently showed less change than those seen on the ischemic side.

Experiment 3. Neurologic Outcome

This experiment was designed to compare N_2O-treated controls with low- and high-dose methohexital and isoflurane at both moderate and severe levels of ischemia. Five experimental groups were studied at each ischemic level: group 1 = 70% or 80% N_2O controls; group 2 = 0.01 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital; group 3 = 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital; group 4 = 1 MAC isoflurane; and group 5 = 2 MAC isoflurane (groups 2–5 received either 70% or 80% N_2).

Neurologic deficits were initially evaluated 3 h after recovery from ischemia and repeated every 8 h for 3 days. Deficits were scored from 0 to 5 by an evaluator blinded to the treatment as follows: 0 = normal, 1 = paw adduction or unusual posture, 2 = circling behavior and unilateral weakness, 3 = stroke-related seizures induced by stimulation, 4 = unstimulated seizures, 5 = death associated with progressive stroke.

Experiment 4. Histopathology

Brain histopathology was performed in separate groups of rats for each drug treatment and ischemic condition

TABLE 2. Mean Blood Pressure, Heart Rate, CBF, and CMR_{O_2} during N_2O, Methohexital, or Isoflurane Anesthesia

	Mean Blood Pressure (mmHg)	Heart Rate (beats/min)	Cortex CBF (ml · 100 g^{-1} · min^{-1})	Subcortex CBF (ml · 100 g^{-1} · min^{-1})	CMR_{O_2} (ml O_2 · 100 g^{-1} · min^{-1})
Methohexital (n = 4)					
70% N_2O (control)	127 ± 12	408 ± 17	285 ± 24	138 ± 12	11.9 ± 1.1
0.01 mg · kg^{-1} · min^{-1}	121 ± 14	413 ± 46	165 ± 20*	115 ± 11	9.1 ± 1.1
0.1 mg · kg^{-1} · min^{-1}	87 ± 2*	388 ± 17	71 ± 12*	63 ± 5*	4.3 ± 0.6*
Isoflurane (n = 4)					
70% N_2O (control)	133 ± 6	363 ± 29	266 ± 30	124 ± 10	11.0 ± 1.3
1 MAC	85 ± 5*	340 ± 11	135 ± 39*	169 ± 40†	8.0 ± 0.6*
2 MAC	76 ± 5*	340 ± 8	108 ± 12*	148 ± 27†	4.4 ± 0.2*

* $P < 0.05$ compared with N_2O control.

† $P < 0.05$ isoflurane compared with equipotent methohexital dose.

(n = 4–6 per group). Twenty-four hours after recovery from the ischemic episode the rats were anesthetized with halothane and killed by transcardial perfusion with 50 ml saline followed by 50 ml of 10% buffered formalin maintained at room temperature. The infusion of both solutions was performed using 50-ml syringes and an 18-G needle placed directly into the left ventricle. Although neuronal damage may progress over several days, 24 h was chosen in this study to obtain histologic tissue under all treatment conditions before the rats died of ischemic injury. Twenty-four hours post ischemia neuronal damage in the caudate is 80–90% complete while cortex and hippocampal damage progress for 72 h after ischemia.[17] The brains were dissected out and immersed in 10% formalin for 1–2 weeks. The forebrain was sliced into coronal blocks, imbedded in parafin wax and 7–8 μm sections, and cut and mounted on slides. These slides were stained using hematoxylin and eosin and examined in a blinded manner by a neuropathologist (C.T.) using light microscopy. Brain histopathology in the ischemic hemisphere was graded on a continuous scale in coronal section at the level of the caudate nucleus with the following general criteria: 0 = no observable neuronal damage, 10 = scattered neuronal death, 20 = moderate focal damage in caudate and cortical areas, 30 = severe damage involving extensive neuronal tissue, and 40 = total infarct.

STATISTICS

Data are reported as mean ± SE. CBF and CMR_{O_2} were analyzed using a two-way analysis of variance with repeated measures for each anesthetic. Differences between means involving multiple tests were compared using Scheffe's tests. Nonparametric neurologic deficit scores were compared between N_2O and either methohexital or isoflurane treatment groups at each ischemic level using Mann-Whitney U tests with a Bonferoni correction. Histopathology was compared between N_2O and methohexital or isoflurane treatment groups at each ischemic level using parametric analysis with Scheffe's tests used to compare means for statistical significance. Comparisons between low-dose methohexital *versus* 1 MAC isoflurane and high-dose methohexital *versus* 2 MAC isoflurane were also made at each ischemic level using the above tests.

Results

CBF AND CMR_{O_2}

Both methohexital and isoflurane decreased blood pressure, cortical CBF, and CMR_{O_2} compared with N_2O controls (table 2). Low-dose isoflurane and methohexital produced 27% and 24% decreases in CMR_{O_2}, respectively, while 2 MAC isoflurane and 0.1 mg · kg^{-1} · min^{-1} methohexital produced 60% and 64% depression, respectively. High-dose methohexital decreased both cortex and subcortex CBF (75% and 55% decrease, respectively) more than 2 MAC isoflurane (60% decrease and 19% increase, respectively) ($P < 0.05$).

ELECTROENCEPHALOGRAM

EEG recordings (figs. 1 and 2) during pre-ischemic control conditions showed that low-dose methohexital (0.01 mg · kg^{-1} · min^{-1}) and 1 MAC isoflurane decreased EEG frequency and increased the voltage amplitude compared with N_2O. These changes were accentuated by ischemia. High-dose methohexital (0.1 mg · kg^{-1} · min^{-1}) and 2 MAC isoflurane produced burst suppression activity before ischemia, although EEG appeared to be more active with methohexital than isoflurane. During ischemia EEG was further suppressed to a state of EEG quiescence with high-dose methohexital and isoflurane.

NEUROLOGIC OUTCOME

Cardiovascular parameters and arterial blood gas tensions during control, ischemic (hypotension), and recovery

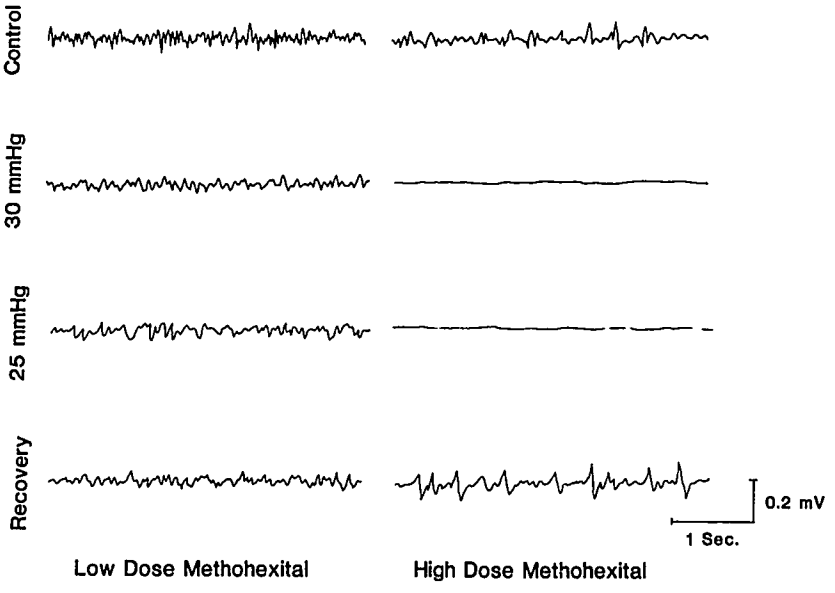

FIG. 1. EEG changes in right (ischemic) hemisphere with low dose (0.01 $mg \cdot kg^{-1} \cdot min^{-1}$) and high dose (0.1 $mg \cdot kg^{-1} \cdot min^{-1}$) methohexital during control state, moderate ischemia (MAP = 30 mmHg, FI_{O_2} = 0.30), severe ischemia (MAP = 25 mmHg, FI_{O_2} = 0.20), and recovery conditions. Baseline EEG was depressed, with burst suppression evident with high dose methohexital. Additional depression occurred with ischemia.

periods are shown in tables 3 and 4. Low-dose methohexital produced little change in control blood pressure compared with N_2O, whereas isoflurane and high-dose methohexital produced cardiovascular depression. Blood gases and *p*H were stable throughout the control, ischemic, and recovery periods. Under baseline (control) conditions, two MAC isoflurane produced significant increases in plasma glucose compared with N_2O. High-dose methohexital resulted in a significantly lower plasma glucose during ischemia compared with ischemic N_2O-treated rats. Neurologic outcome following moderate and severe ischemia is shown in figure 3. Compared with 70% N_2O both low- and high-dose methohexital and 1 and 2 MAC isoflurane significantly improved outcome following moderate ischemia. Following severe ischemia, only 2 MAC isoflurane improved outcome.

HISTOPATHOLOGY

Histopathologic damage seen following ischemia is shown in figure 4. Following moderate ischemia with both low- and high-dose methohexital and 1 and 2 MAC isoflurane neurohistopathology scores were decreased compared with N_2O-treated rats ($P < 0.05$). Isoflurane 2 MAC was associated with significantly less neuronal damage than that during high-dose methohexital ($P < 0.05$). Following

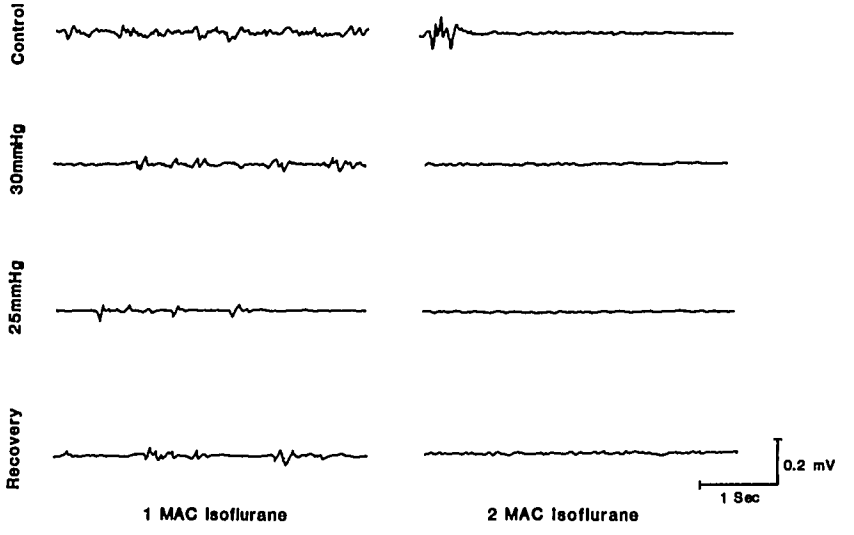

FIG. 2. EEG changes in right (ischemic) hemisphere with 1 and 2 MAC isoflurane during baseline control state, moderate ischemia (MAP = 30 mmHg, FI_{O_2} = 0.30), severe ischemia (MAP = 25 mmHg, FI_{O_2} = 0.20), and recovery conditions. Baseline EEG was depressed with burst suppression present at 2 MAC. Ischemia further depressed EEG, with little return of activity during the recovery period.

TABLE 3. Mean Blood Pressure, Heart Rate, Plasma Glucose, and Blood Gas Tensions during Moderate Ischemia

Treatment	Mean Blood Pressure (mmHg)	Heart Rate (beats/min)	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	*p*H	Plasma Glucose (mg/100 ml)
70% N_2O						
Control (n = 9)	132 ± 2	385 ± 5	36.2 ± 1.2	135 ± 5	7.40 ± 0.2	174 ± 7
Hypotension	30 ± 1*	397 ± 6	36.7 ± 1.3	151 ± 6	7.37 ± 0.2	407 ± 35*
Recovery	118 ± 3	373 ± 8	39.3 ± 1.5	129 ± 6	7.38 ± 0.02	215 ± 26
0.01 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital						
Control (n = 8)	125 ± 4	440 ± 14	38.8 ± 1.0	138 ± 5	7.43 ± 0.01	177 ± 5
Hypotension	31 ± 1*	410 ± 19	34.5 ± 1.0	148 ± 6	7.42 ± 0.02	400 ± 43*
Recovery	106 ± 5	445 ± 14	38.3 ± 0.4	142 ± 5	7.41 ± 0.02	260 ± 52*
0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital						
Control (n = 10)	104 ± 4†	440 ± 10	39.3 ± 0.9	136 ± 9	7.43 ± 0.01	159 ± 7
Hypotension	30 ± 1*	336 ± 9*	43.4 ± 1.6	135 ± 10	7.43 ± 0.03	232 ± 28*†
Recovery	99 ± 5†	422 ± 12	40.1 ± 0.7	125 ± 11	7.45 ± 0.01	144 ± 26†
1 MAC isoflurane						
Control (n = 10)	101 ± 7†	368 ± 15	42.8 ± 1.4	143 ± 9	7.40 ± 0.01	180 ± 9
Hypotension	31 ± 1*	291 ± 12*	37.2 ± 2.2	136 ± 13	7.37 ± 0.02	292 ± 19*†
Recovery	98 ± 6*†	314 ± 10	41.0 ± 2.2	140 ± 5	7.38 ± 0.02	199 ± 14
2 MAC isoflurance						
Control (n = 10)	69 ± 2†	342 ± 8	37.6 ± 2.1	148 ± 2	7.40 ± 0.1	258 ± 16†
Hypotension	30 ± 1*	337 ± 9	39.3 ± 0.8	142 ± 4	7.36 ± 0.2	477 ± 15*†
Recovery	91 ± 4*†	382 ± 7	39.6 ± 0.9	148 ± 12	7.39 ± 0.01	352 ± 12*†

Hypotension indicates 30-min period of ischemia in each group.
* $P < 0.05$ compared with group control.
† $P < 0.05$ compared with N_2O.

TABLE 4. Mean Blood Pressure, Heart Rate, Plasma Glucose, and Blood Gas Tensions during Severe Ischemia

Treatment	Mean Blood Pressure (mmHg)	Heart Rate (beats/min)	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	*p*H	Plasma Glucose (mg/100 ml)
80% N_2O						
Control (n = 10)	127 ± 3	373 ± 4	39.2 ± 1.2	88 ± 3	7.41 ± 0.02	167 ± 8
Hypotension	25 ± 1*	384 ± 5	35.4 ± 1.1	111 ± 4*	7.38 ± 0.02	395 ± 18*
Recovery	132 ± 6	382 ± 6	43.2 ± 1.6	79 ± 3	7.36 ± 0.02	195 ± 12
0.01 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital						
Control (n = 8)	120 ± 2	466 ± 11	37.1 ± 1.7	77 ± 1	7.41 ± 0.02	167 ± 8
Hypotension	26 ± 1*	473 ± 15	35.3 ± 1.3	94 ± 3	7.38 ± 0.01	315 ± 28*
Recovery	96 ± 9†	483 ± 16	38.8 ± 1.1	69 ± 4	7.41 ± 0.02	233 ± 36
0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital						
Control (n = 8)	145 ± 1	400 ± 8	34.0 ± 1.1	74 ± 3	7.47 ± 0.01	160 ± 5
Hypotension	25 ± 1*	330 ± 6*	38.3 ± 1.4	90 ± 4	7.40 ± 0.02	261 ± 30*†
Recovery	84 ± 6*†	385 ± 6	38.6 ± 1.5	70 ± 5	7.42 ± 0.02	172 ± 23
1 MAC isoflurane						
Control (n = 10)	103 ± 4†	338 ± 14	36.6 ± 2.1	81 ± 4	7.45 ± 0.01	176 ± 4
Hypotension	25 ± 1*	292 ± 20	35.1 ± 1.2	96 ± 4	7.39 ± 0.01	330 ± 22*
Recovery	112 ± 5†	320 ± 11	36.2 ± 0.8	78 ± 10	7.39 ± 0.01	195 ± 9
2 MAC isoflurane						
Control (n = 12)	66 ± 2†	316 ± 10	37.4 ± 1.8	80 ± 3	7.43 ± 0.01	240 ± 19†
Hypotension	25 ± 1*	333 ± 13	35.7 ± 1.6	89 ± 4	7.37 ± 0.01	377 ± 22*
Recovery	83 ± 4†	343 ± 9	39.1 ± 1.3	77 ± 4	7.40 ± 0.01	277 ± 25†

Hypotension indicates the 30-min period of ischemia in each group.
* $P < 0.05$ compared with group control.
† $P < 0.05$ compared with N_2O.

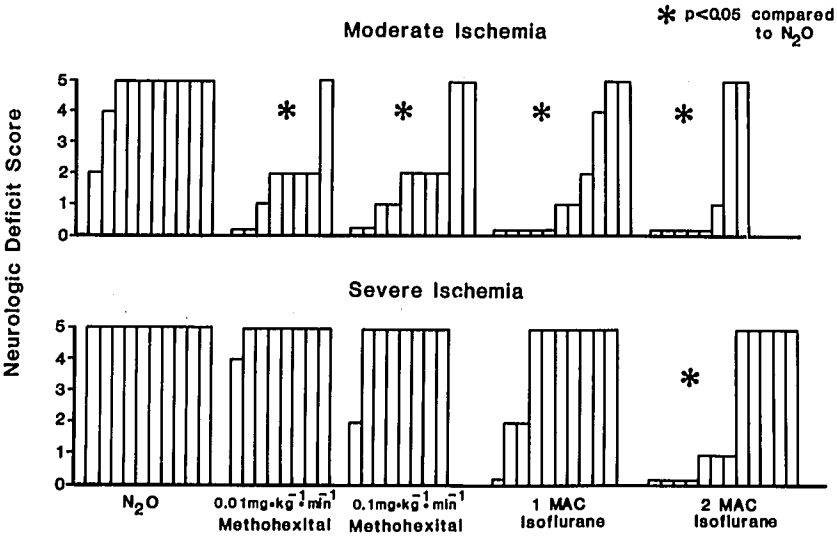

FIG. 3. Neurologic deficit scores following moderate ischemia (MAP = 30 mmHg, $F_{I_{O_2}}$ = 0.30) and severe ischemia (MAP = 25 mmHg, $F_{I_{O_2}}$ = 0.20). Treatment groups include N_2O (70–80%), low and high dose methohexital, and 1 or 2 MAC isoflurane. Each bar represents the neurologic score for one rat. Significance value (*) indicates difference *versus* N_2O at each ischemic level ($P < 0.05$). There was no statistical difference between low dose methohexital *versus* 1 MAC isoflurane or high dose methohexital *versus* 2 MAC isoflurane at either ischemic level.

severe ischemia 2 MAC isoflurane produced a significantly better neurohistology score compared with that during both N_2O or high-dose methohexital.

Discussion

These results show that methohexital and isoflurane produced similar dose-related decreases in CMR_{O_2} and significant improvement in outcome following moderate ischemia, whereas only 2 MAC isoflurane produced a better outcome following severe ischemia. These results support previous work showing that isoflurane and barbiturates protect the brain from ischemia.[1–3,6–8,10] Our data also suggest that cerebral protection is mediated, in part, by cerebral metabolic depression. However, other factors may also be important because comparable CMR_{O_2} depression by high-dose methohexital and 2 MAC isoflurane did not produce the same outcome following severe ischemia.

Some technical issues need to be addressed. First is the recovery from anesthesia. Anesthetic and vecuronium administration was discontinued in each treatment group so that the rat extubated its trachea itself approximately 45 min after the end of ischemia. This required ending the anesthetic treatment sooner for methohexital compared with isoflurane and N_2O-treated rats. The blood that was withdrawn to produce hypotension was maintained at room temperature and was not warmed prior to reinjection; however, the blood was reinfused slowly through the subclavian venous catheter, and all rats were maintained at 37° C until the time of extubation. These procedures were designed to produce similar recovery paradigms in each treatment group and to minimize possible differences due to body temperature or ventilation. It also allowed similar intubation and ventilation support times for each anesthetic and dose since recovery from methohexital required more time.

Another question is what type of ischemia this model

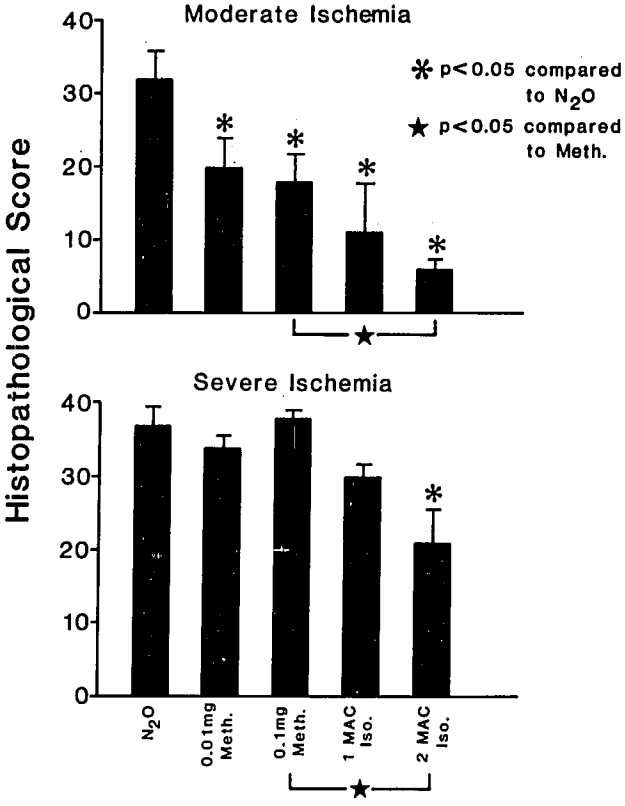

FIG. 4. Histopathology scores with N_2O, methohexital (0.01 and 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$), and isoflurane (1 and 2 MAC) following moderate ischemia (MAP = 30 mmHg, $F_{I_{O_2}}$ = 0.30) and severe ischemia (MAP = 25 mmHg, $F_{I_{O_2}}$ = 0.20). The star indicates a significant difference in comparing 2 MAC iso *versus* 0.1 $mg \cdot kg^{-1} \cdot min^{-1}$ methohexital.

actually represents. It is apparent from previous measurements of CBF during ischemia that this model produces hemispheric incomplete ischemia.[18] However, we would not expect the degree of ischemia to be homogeneous throughout the hemisphere but that it would be worst in watershed regions of the caudate and neocortex between major arterial blood supplies. This may in part explain the selectivity of the caudate and approximating neocortical tissue to ischemic damage in our studies. This is in contrast to brain regions, such as the insular cortex and thalamus, which show the most damage following challenges of complete cerebral ischemia in monkeys.[19]

Barbiturates have been shown to reduce infarct size and improve biochemical and neurologic outcome from ischemia in both clinical and animal studies.[9,20–23] Although it was originally thought that barbiturates may improve recovery from complete ischemia,[24,25] this concept has not been supported.[26,27] Barbiturates are effective in providing brain protection from incomplete ischemia, where blood flow to neurons is present but inadequate to prevent ischemia.[28,29] Under these conditions barbiturates depress neuronal electrical activity and decrease CMR_{O_2} approximately 50%, thereby reducing neuronal oxygen and substrate needs.[30,31]

In a manner similar to barbiturates, isoflurane depresses EEG to burst suppression, decreases brain metabolism by approximately 50%, and therefore has been proposed to protect the brain from incomplete ischemia.[4,5,32,33] As with barbiturates, this protection is probably related to the ability of isoflurane to decrease neuronal metabolic demand.[32,33] This is consistent with our results. Isoflurane produced dose-related decreases in CMR_{O_2} and 2 MAC isoflurane produced significant protection following not only moderate but also severe ischemia. It is unclear why high-dose methohexital, which decreased CMR_{O_2} similar to that produced by 2 MAC isoflurane, did not provide as good neurologic outcome and histopathology scores following severe ischemia. Yatsu *et al.*[34] previously reported that methohexital protects the brain from ischemia. Conversely, Todd *et al.*[35] showed that following prolonged methohexital administration (12–24 h), three of eight patients had refractory postoperative seizures. They suggest that methohexital, a chemical convulsant, may have produced the seizures. Alternatively, the seizures may be due to acute barbiturate withdrawl because they all occurred postoperatively. Although none of these patients had new neurologic deficits following surgery, Todd *et al.*[35] suggested that these postanesthetic seizures could lead to a worse outcome following ischemia. In our study the worse neurologic outcome and histopathology seen with high-dose methohexital *versus* 2 MAC isoflurane may be related to the more active EEG, even though CMR_{O_2} was similar between the two anesthetics. These differences may be a reflection of differences in synaptic activity that occur without a difference in CMR_{O_2} depression. Such an effect may be related to neurotransmitter release and synaptic activity rather than cerebral metabolic depression.

An additional explanation for the difference in neurologic outcome may be due to the cerebrovascular effects of these two drugs. It has been suggested that drugs that produce cerebrovasoconstriction (*i.e.*, methohexital) may be beneficial during ischemia by shunting blood flow to the ischemic tissue. However, if total cerebral blood flow is significantly depressed, this beneficial effect may not exist. Conversely, drugs that produce cerebrovasodilation (*i.e.*, isoflurane) may be either helpful during ischemia by increasing total CBF or harmful by diverting blood away from the maximally dilated ischemic area. Our results do not support the concept that the cerebrovasodilation effect of isoflurane worsens outcome from ischemia by stealing blood flow from the ischemic zone. Previous work with this model[18] has shown that CBF during ischemia is probably blood pressure dependent and that the vasoactive effects of drugs are unimportant in this model. However, additional measures of CBF must be made during ischemia to determine what effect these drugs have, if any, on cerebral blood flow redistribution during incomplete hemispheric ischemia.

Whether isoflurane provides as much protection as that provided by barbiturates from incomplete ischemia is controversial. Nehls *et al.*[9] reported that isoflurane anesthesia produced a worse neurologic outcome and larger infarcts following middle cerebral artery occlusion in baboons compared with that following equipotent doses of thiopental as measured by EEG suppression. However, in that study it was necessary to treat the isoflurane anesthetized baboons with vasopressor drugs and the thiopental anesthetized animals with vasodilators during ischemia in an only partially successful attempt to control blood pressure. This raises the possibility that either blood pressure differences or the vasoactive drugs may have had a role in affecting outcome from ischemia. Milde *et al.*[10] repeated those studies in the pigtail monkey, which has cardiovascular responses to isoflurane and thiopental that are similar to human responses. Their results show that isoflurane and thiopental produced similar neurologic outcomes from ischemia, although they were not compared with a control treatment. Our results agree more closely with those of Milde *et al.*[10] in that we observed that both isoflurane and methohexital produce similar neurologic outcomes and protect the brain from ischemia compared with N_2O controls.

It is well accepted that even moderately elevated blood glucose concentration adversely affects outcome from incomplete ischemia.[36–38] This may be due to increased

glucose availability and lactate formation in ischemic regions that accelerate neuronal death, possibly by decreasing intracellular *p*H. Measurement of plasma glucose in our study showed increases during the ischemic period in all anesthetic groups, consistent with a stress response. We also showed higher baseline plasma glucose levels during 2 MAC isoflurane, which is consistent with other reports.[32,39] Elevation of plasma glucose would suggest that the 2 MAC isoflurane group should have a worse outcome associated with brain tissue lactate production, whereas high-dose methohexital would produce less brain acidosis. The fact that outcome was better with 2 MAC isoflurane than with methohexital would suggest that plasma glucose was not the key factor in determining outcome in these studies. Outcome may have been further improved in isoflurane-treated rats if plasma glucose were not allowed to increase before the start of ischemia.

From this study we conclude that both methohexital and isoflurane improve outcome from incomplete ischemia in rats compared with that during N_2O. Two MAC isoflurane and high-dose methohexital produce comparable decreases in CMR_{O_2}, but isoflurane resulted in better protection from severe ischemia as measured by neurologic outcome and cerebral histopathology. Factors that may explain superior brain protection with isoflurane include a possible difference in synaptic transmission during deep anesthesia and/or possible differences in CBF flow or redistribution produced by the two anesthetics.

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