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Nitrous Oxide Does Not Alter Infarct Volume in Rats Undergoing Reversible Middle Cerebral Artery Occlusion

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This experiment was designed to determine if nitrous oxide alters neurologic and pathologic outcome from temporary focal cerebral ischemia in spontaneously hypertensive rats deeply anesthetized with a barbiturate. Two groups of rats were given intravenous methohexital such that a stable EEG pattern of burst suppression was achieved. In one group of rats (n = 11), the lungs were mechanically ventilated with 70% N_2O/30% O_2, and in the other group (n = 10), ventilation was done with 70% nitrogen/30% O_2. The middle cerebral artery was then occluded for 2 h, during which time mean arterial pressure, blood gases, hematocrit, plasma glucose, and head temperature were held constant between groups. The total doses of methohexital administered were similar in both groups as were the plasma methohexital concentrations immediately prior to onset of ischemia. After reperfusion of the middle cerebral artery, the animals were allowed to awaken. Neurologic evaluations were performed prior to ischemia and at 24 and 96 h postischemia. Cerebral infarct volume was measured at 96 h postischemia using triphenyl tetrazolium chloride staining and computer imaging techniques. There were no neurologic differences between the N_2O and nitrogen groups at any experimental interval although both groups exhibited deficits at both 24 and 96 h postischemia relative to preischemic values. The two groups also had nearly identical cerebral infarct volumes (N_2O = 231 ± 97 mm^3; nitrogen = 226 ± 75 mm^3; mean ± SD). In a subset of identically anesthetized rats not undergoing ischemia (70% N_2O: n = 5; 70% N_2: n = 5), the cerebral metabolic rate for glucose (CMR_{glu}) was autoradiographically assessed in nine anatomic structures with the [^{14}C]-2-deoxyglucose technique. No differences between anesthetic groups were observed in any structure. (CMR_{glu} values ranged between 12–33 μmol/100 g/min.) The authors concluded that N_2O has no metabolic stimulatory effect in rats deeply anesthetized with methohexital. In addition, the presence or absence of N_2O in the respiratory gas mixture (given a constant FI_{O_2}) does not alter outcome from focal ischemia in rats undergoing deep barbiturate anesthesia. (Key words: Anesthetics, gases: nitrous oxide. Anesthetics, volatile: methohexital. Brain: infarction; ischemia; metabolism. Rat.)

NITROUS OXIDE remains a commonly administered anesthetic agent during neurosurgical procedures and in experimental preparations used for the study of cerebral ischemia. In particular, several studies evaluating effects of barbiturates on outcome from cerebral ischemia have co-administered nitrous oxide with the barbiturate during the ischemic insult.[1–3] Although the effects of N_2O on cerebral blood flow (CBF) may be substantial,[4–6] effects on cerebral metabolic rate generally have been considered modest.[7,8] Therefore, if the mechanism by which barbiturates reduce ischemic brain damage is related to a reduction in cerebral metabolic rate, the use of N_2O would seem inconsequential.

Other laboratory evidence disputes this assumption. First, under some conditions, N_2O has been demonstrated to cause substantial increases in cerebral metabolic rate.[4,9] Secondly, it has been reported that when mice were exposed to a hypoxic gas mixture, prolongation of survival time induced by pretreatment with intraperitoneal thiopental was abolished if the hypoxic gas mixture contained 50% N_2O.[10] This observation prompted a reevaluation of a large number of previous studies investigating the efficacy of barbiturates in reducing ischemic/hypoxic brain damage. Such studies were segregated according to whether N_2O was present or absent in the respiratory gas mixture during the insults. In most instances where barbiturates were shown to protect, N_2O was absent. In contrast, in those studies where N_2O was co-administered with barbiturates, protection only was observed infrequently. This observation is remarkable because this predictor of barbiturate protection was independent of the more classic predictor, *i.e.*, incomplete *versus* complete cerebral ischemia.

This study was designed to directly test the hypothesis that N_2O alters outcome from a focal ischemic insult occurring during deep barbiturate anesthesia. Rats were anesthetized with methohexital in sufficient doses to produce EEG burst suppression. One half of the animals were concomitantly administered N_2O while the remainder were not. After 2 h of reversible middle cerebral artery occlusion and a 4-day recovery interval, the resultant cerebral infarct volumes and neurologic outcome were measured and compared between the two groups.

Methods

SURGICAL PREPARATION

This study was approved by the University of Iowa Animal Care and Use Committee. At 13 or 14 weeks of age, male spontaneously hypertensive rats (SHR; Harlan, Indianapolis, IN) were fasted from food for 12–16 h prior to the experiment but allowed free access to water. All animals were then weighed and anesthetized with 3–4%

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halothane in 30% O_2/balance nitrogen. The trachea of each rat was intubated, and the lungs were mechanically ventilated at a rate of 60 breaths per min and a tidal volume of 2.5 ml. The tail artery was catheterized *via* surgical incision for measurement of blood pressure and blood gases. *Via* a transverse neck incision, the right internal jugular vein was cannulated with a Silastic® catheter. Body temperature was monitored *via* a rectal probe and was servo-controlled to 37.0° C by surface heating or cooling. A left subtemporal craniectomy was then performed. Aided by an operating microscope, the dura and arachnoid were opened, and the left middle cerebral artery (MCA) was identified.[11] The MCA was then loosely encircled with a 10–0 suture just distal to the lenticulostriate artery. Bilateral EEG needle electrodes were placed adjacent to the parietal bones, and a reference needle electrode was placed subcutaneously in the animals' snout. An overall interval of 1 h (from induction of anesthesia) was allowed to complete surgical preparation. Pancuronium was given as a 0.3 mg iv bolus, with subsequent doses of 0.1 mg given as necessary for control of ventilation. Following preparation, all rats received 50 IU of iv heparin.

Halothane was discontinued after surgical preparation. With the EEG being continuously recorded (Grass Model 79D, Quincy, MA), an intravenous infusion of 2.5% methohexital (500 mg dissolved in 20 ml of 0.9% NaCl) was begun. Initially, the solution was administered at 0.8 ml/h until EEG burst suppression was achieved. Subsequently, the infusion rate was titrated to maintain a stable EEG interburst interval of 5–15 s, typically requiring an infusion rate of 0.4–0.6 ml/h. The animals were then randomly assigned to one of two groups. In one group, the inspiratory gas mixture (O_2 in N_2) was adjusted to produce an FI_{O_2} of 0.3. In the other group, nitrogen was replaced with N_2O, the FI_{O_2} again being adjusted to 0.3 as determined with an O_2 analyzer. Ventilation was adjusted to ensure normocapnia. Mean arterial blood pressure was controlled within the range of 90–100 mmHg by infusion of donor rat blood or bleeding as necessary. The above sequence was completed within a 45-min interval after completion of surgical preparation. Venous blood was then withdrawn (1 ml) for later determination of hematocrit, plasma glucose (glucose oxidase method, YSI Model 27, Yellow Springs, OH), and methohexital concentration (gas chromatography).

Thirty minutes later (75 min after starting the methohexital), all rats underwent a 2-h interval of MCA occlusion accomplished by tightening of the ligature previously placed around the MCA. Absence of flow was visually verified by an observed blanching of the vessel distal to the occlusion. The wound was then loosely closed. During this interval, Pa_{O_2}, Pa_{CO_2}, *p*H, and Hct were determined at hourly intervals. At the mid-point of the ischemic interval, additional heparin (35 IU) was administered iv. In addition, a 25-G needle thermistor (YSI Model 524) was inserted into the wound in contact with the temporal bone, in the direct vicinity of the craniectomy site, allowing pericranial temperature to be servo-regulated at 37.0° C by surface heating or cooling throughout the ischemic interval.

After 2 h, the wound was reopened, and the ligature was permanently removed from the MCA. The methohexital infusion was then discontinued and the N_2O, if present, was replaced with nitrogen. Reperfusion was verified by observation that the vessel filled with blood at the ligature site. The temporalis muscle was approximated with suture, and the scalp wound was closed. Just before recovery, the jugular and arterial catheters were removed, and the respective wounds were closed. The animals were allowed to awaken, and when breathing spontaneously, the tracheas were extubated. The rats were then placed into a warmed, oxygen-enriched environment for 1–2 h and later returned to their cages.

Neurologic Evaluation

All recovering rats underwent a three-part series of neurologic function tests both prior to the surgical procedure and at 24 and 96 h postischemia. In part 1, motor function was performed as described by Combs and D'Alecy.[12] Briefly, the rats were placed on a 29 × 30-cm screen (grid size, 0.6 × 0.7 cm) that could be rotated from 0° (horizontal) to 90° (vertical). The rat was placed on the screen when horizontal, and the screen was then rotated into the vertical plane. The duration of time that the rat was able to hold onto the vertical screen was recorded to a maximum of 15 s (allowing a total of 3 points). Next, the rat was placed at the center of a horizontal wooden rod (1 inch diameter), and the duration that the animal was able to remain balanced on the rod was recorded to a maximum of 30 s (allowing a total of 3 points). Finally, a prehensile-traction test was administered. The duration the animal was able to cling to a horizontal rope was recorded to a maximum of 5 s. From these three tests, a total motor score (9 possible points) was computed.

Part 2 of neurologic testing involved application of the neurologic scoring system described by Bederson *et al.*[13] In this case, the animals were scored on a scale of 0–3: 0 = no observable deficit; 1 = forelimb flexion; 2 = decreased resistance to lateral push without circling; and 3 = same behavior as 2, with circling.

Finally, in part 3, the animals were evaluated using an open-field test that was intended to measure the animals potential for exploratory activity.[14] The rats were placed in a wooden box with a square floor measuring 90 × 90 cm. The floor was painted with a grid, each square being 15 × 15 cm in area (*i.e.*, a total of 36 squares). The animals

were observed over a 5-min period, and the number of squares the animal's snout entered and the number of times the animal raised up on its hindlimbs was recorded.

All tests were performed by a single experimenter (RR) who was blinded to the experimental condition of the animal. In every case, evaluations were performed in the same darkened quiet room after a brief period of acclimation.

Quantification of Infarct Volume

Following the 96-h neurologic evaluation, the animals were re-weighed and anesthetized with 4% halothane in O_2. They were then decapitated and the brains rapidly removed. Each brain was refrigerated at 6° C for 10 min to facilitate sectioning. Using a brain dissection block (Zivic-Miller, Zelienople, PA), the brains were cut into 2-mm thick coronal sections over the entire rostral caudal extent of the forebrain. The sections were then immersed for 30 min in a 2% solution of triphenyl tetrazolium chloride (Sigma) buffered to a *p*H of 7.4 and kept at 37° C. This water-soluble compound is enzymatically converted in intact mitochondria to a fat soluble formazan compound that stains viable tissue crimson red while infarcted tissue fails to stain and appears white (fig. 1).[15,16] The sections were then stored in a 4% formalin solution for later analysis of infarct volume.

Infarct volume was measured by placing each section into a shallow saline bath under a television camera interfaced with a Digital Microvax II computer and a Gould image analyzer. The image of each section was digitized according to optical density (reflectance), and the data were stored as a matrix of pixel units. For each tissue section, the pixel units were calibrated to give values as mm^2. The digitized image was then displayed on a video terminal. With the experimenter blinded to the anesthetic condition, the infarct border was outlined using an operator-controlled cursor, and the infarct area was computed. In addition, the overall area for both the ipsilateral and contralateral hemispheres was determined. For each rat, infarct volume was computed as the running sum of infarct area times the 2-mm thickness of each section over the rostral-caudal extent of the forebrain. The total hemispheric volumes were similarly assessed.

Determination of Cerebral Metabolic Rate

Ten additional age-matched and fasted SHR rats were surgically prepared in a manner identical to that described above with one exception: in these animals, scalp and temporalis flaps were raised but the skull was not violated. After surgical preparation, halothane was discontinued, and the animals were randomly assigned to one of the two anesthetic groups (n = 5 for each) described above (*i.e.*, intravenous methohexital infusion with or without N_2O in the inspiratory gas mixture), with depth of anesthesia (*i.e.*, methohexital infusion rate) regulated to produce and maintain an EEG pattern of burst suppression. Identical time intervals were allowed for surgery and anesthetic stabilization as were described for the recovery animals above. At the time when the MCA would have been occluded, 100 μCi/kg of ^{14}C-labeled 2-deoxyglucose ([^{14}C]2-DG, specific activity of 60 mCi/mmol, American Radiolabeled, Inc., St. Louis, MO) was infused iv over 45

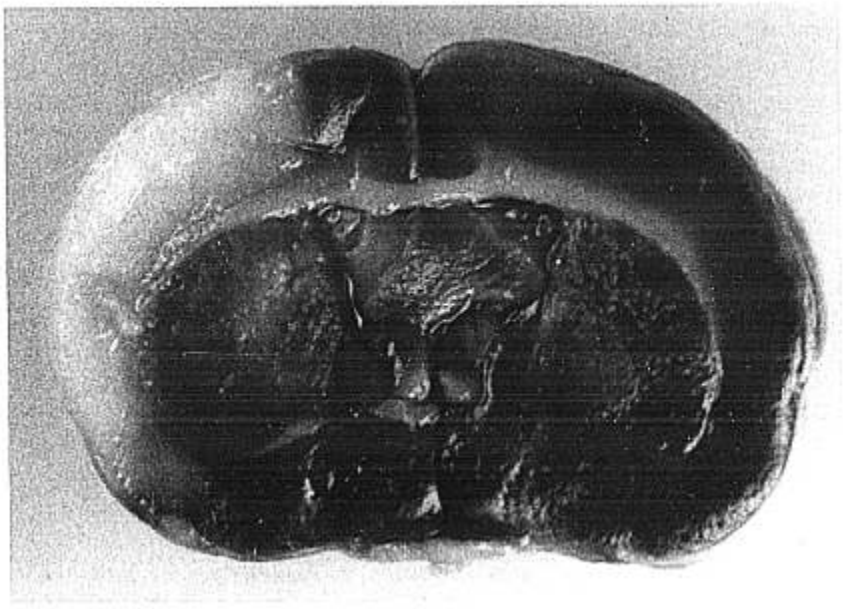

FIG. 1. Coronal section of a rat brain evaluated 96 h after a 2-h interval of middle cerebral artery occlusion occurring during deep methohexital anesthesia with 70% N_2O/30% O_2. After sectioning, the fresh tissue was immersed in a 37° C solution of triphenyl tetrazolium chloride (2%) for 30 min. The unstained (white) area represents infarcted tissue.

s. Over the subsequent 45 min, 14 timed arterial blood samples were taken for later determination of serial plasma glucose concentrations and radioactivities.

The brains were then excised and frozen immediately in n-pentane (−55° C). The frozen brains were coronally cut on a freezing microtome in 20-μm thick sections at standardized anatomic intervals. The sections were mounted on glass slides and exposed along with autoradiographic standards to Kodak SB5 autoradiographic film for 7 days. The images were digitized according to optical density with a scanning microdensitometer system. Using an operator-controlled cursor, anatomic regions of interest were outlined on at least two tissue sections for each region. Recalculation of the cerebral metabolic rate for glucose (CMR_{glu}) values for each region of interest used the equations developed by Sokoloff *et al.*[17] (lumped constant = 0.483) with mathematical operations performed using a quantitative glucose utilization program (A. Toga, Washington Univ., St. Louis, MO) run on a Digital Microvax II computer.

Statistical Analysis

Physiologic values were compared between the N_2O and nitrogen groups in both the recovery and CMR_{glu} experiments by the unpaired Student's *t* test. Infarct volumes and CMR_{glu} values were also compared by Student's *t* test. The total motor score and the neurologic grades were compared between the nitrogen and N_2O groups with a nonparametric two-sample linear rank test.[18] The same scores were compared within groups over time by the Cochran-Mantel-Haenszel test of time marginal homogeneity.[19] Exploratory activity parameters were compared between groups over time by a repeated measures analysis of variance with posthoc testing (Newman-Keuls *t* test) for within group differences where appropriate. Statistical significance was assumed when $P < 0.05$. Parametric values are reported as mean ± SD.

TABLE 1. Physiologic Values for Methohexital-Anesthetized Rats Undergoing Ischemia in the Presence or Absence of 70% Nitrous Oxide in the Inspiratory Gas Mixture

Value	Nitrogen (n = 10)	Nitrous Oxide (n = 11)
Pa_{O_2} (mmHg)	107 ± 9	102 ± 6
Pa_{CO_2} (mmHg)	39.1 ± 1.1	38.9 ± 1.4
Arterial pH	7.36 ± .04	7.36 ± .02
MAP (mmHg)	95 ± 2	96 ± 3
Hematocrit (%)	46 ± 3	47 ± 2
Plasma glucose (mg/dl)	105 ± 15	107 ± 8
Body weight (g)		
Preischemia	289 ± 15	280 ± 29
96 h postischemia	262 ± 19	259 ± 26
Rectal temperature (° C)	37.0 ± 0.1	37.0 ± 0.1
Skull temperature (° C)	37.0 ± 0.1	37.0 ± 0.1
Estimated blood loss (ml)	3 ± 1	3 ± 1
Blood given (ml)	1 ± 1	1 ± 1

Values (means ± SD) taken from blood samples were those obtained immediately prior to onset of ischemia. There were no differences between groups.

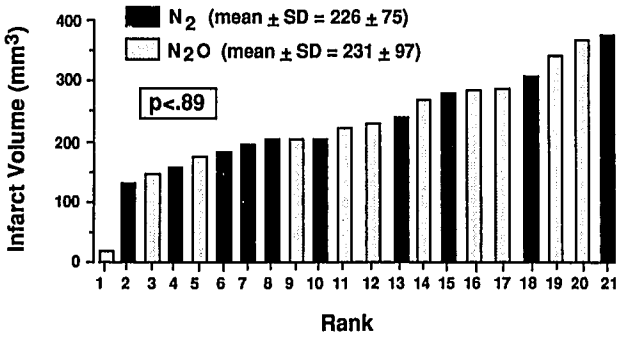

FIG. 2. Cerebral infarct volume (cubic millimeters) for individual rats as a function of whether nitrous oxide (70%) or nitrogen (70%) was being present during a 2-h interval of reverisble middle cerebral artery occlusion. All rats were anesthetized with a methohexital infusion in a dose sufficient to produce an EEG pattern of burst suppression. There was no difference between groups.

Results

Physiologic values are presented in table 1 for animals undergoing MCA occlusion and recovery. No significant differences between experimental groups were observed for Pa_{O_2}, Pa_{CO_2}, arterial *p*H, rectal or skull temperature, plasma glucose, Hct, or mean arterial pressure either immediately prior to ischemia or at 1 or 2 h after onset of ischemia. The total doses of methohexital delivered to produce and sustain EEG burst suppression for 2 h were similar between recovery groups (nitrogen = 45 ± 6 mg; N_2O = 50 ± 5 mg). This was reflected by the plasma methohexital concentrations present immediately prior to the onset of ischemia that were also similar between groups (15.6 ± 5.6 μg/ml for the N_2O group *vs.* 17.0 ± 4.8 μg/ml for the N_2 group).

A total of 28 rats were entered into the outcome study. Two rats in each group died during the 4-day recovery interval. Overt seizure activity was not observed in any of these animals, and the cause of death was not determined. In two rats from the nitrogen group and one rat from the N_2O group, specimen preparation was inadequate for determination of infarct volume. Cerebral infarct volumes for the remaining rats are depicted in figure 2. Infarct volume for the N_2O group (n = 11) was 231 ± 97 mm^3 as opposed to 226 ± 75 mm^3 in the nitrogen group (n = 10). These values were not significantly different ($P < 0.89$). Hemispheric volumes (either contralateral or ipsilateral) were also not different between groups.

No differences were observed between the two groups for any of the three neurologic tests at any interval of observation. The total motor score (with a maximum value of 9) is given in figure 3 for each animal. While both groups had similar values at each observation interval, a deficit was observed at 24 h postischemia in both groups ($P < 0.05$). By 96 h, the total motor score was not different from preischemia values in the N_2O group while in the nitrogen group a deficit persisted ($P < 0.05$). Figure 4 illustrates the neurologic deficit scores. Again, values were without differences between groups at each interval. For this test, both groups demonstrated significantly reduced scores at 24 h that persisted through the 96-h interval ($P < 0.05$). Values from the open-field test are presented in table 2. Reduced activity was observed at both 24 and 96 h postischemia when compared to pre-ischemic values ($P < 0.05$), although at no point was there a difference between groups.

Table 3 depicts physiologic values for the rats entered into the CMR_{glu} study. There were no differences between groups for any parameter. Regional CMR_{glu} values for these groups are presented in table 4. No differences between groups were observed in any of the nine structures evaluated

Discussion

Numerous investigators seeking to increase tolerance of cerebral tissue to ischemia have used deep barbiturate anesthesia to maximize pharmacologic CMR depression.[1-3,20-26] The logic supporting this approach is that the initial trigger for ischemic cell death is an imbalance between metabolic supply and demand. If supply cannot be improved, then, in some instances, a reduction in demand might allow a prolonged tolerance of cerebral tissue to ischemia.

Barbiturates are often thought to reduce metabolic requirements by suppression of electrical activity in the brain. This is derived from the observation that increasing doses of barbiturates are associated with a decrease in CMR until the EEG becomes isoelectric, whereupon no further reduction in CMR is observed.[27] Thus, if the ischemic insult is sufficiently severe as to produce isoelectricity, there would be no potential mechanism for the drug to exert a protective effect simply because there would be no electrical activity left to suppress.[3] This hypothesis is strongly supported by laboratory evidence. In numerous studies examining conditions of severe global ischemia (*i.e.*, ischemia severe enough to cause EEG silence), barbiturates have failed to protect.[1,22,23] In contrast, when ischemic conditions are mild enough to allow persistent EEG activity, barbiturates have repeatedly been found to improve outcome.[20,21,24-26] In particular, we recently demonstrated, in a preparation identical to that described

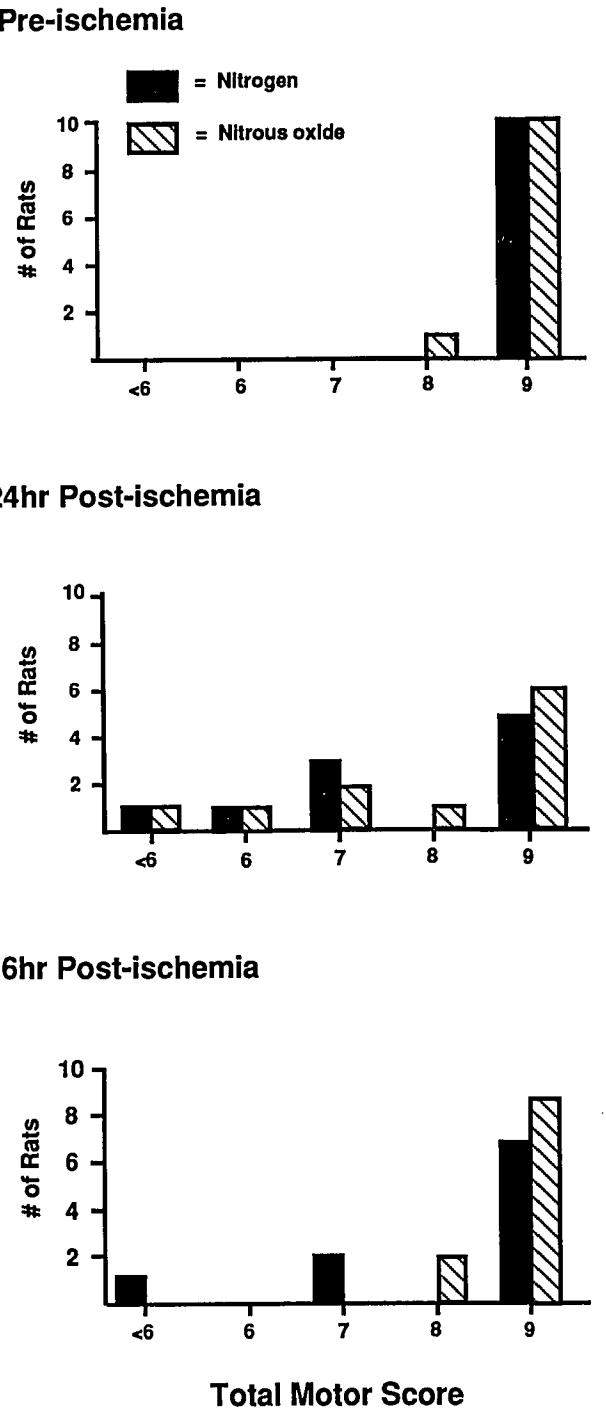

FIG. 3. Total motor scores according to the method of Combs and D'Alecy[12] as a function of experimental interval and of whether nitrous oxide (70%) or nitrogen (70%) was administered during 2 h of middle cerebral artery occlusion in rats receiving deep methohexital anesthesia. A score of 9 indicates normal behavior. No difference between groups was observed at any interval. In both groups the scores were reduced at 24 h postischemia ($P < 0.05$) relative to preischemia values. At 96-h postischemia, the scores in the nitrous oxide group were not different from preischemia values, while in the nitrogen group a deficit persisted ($P < 0.05$).

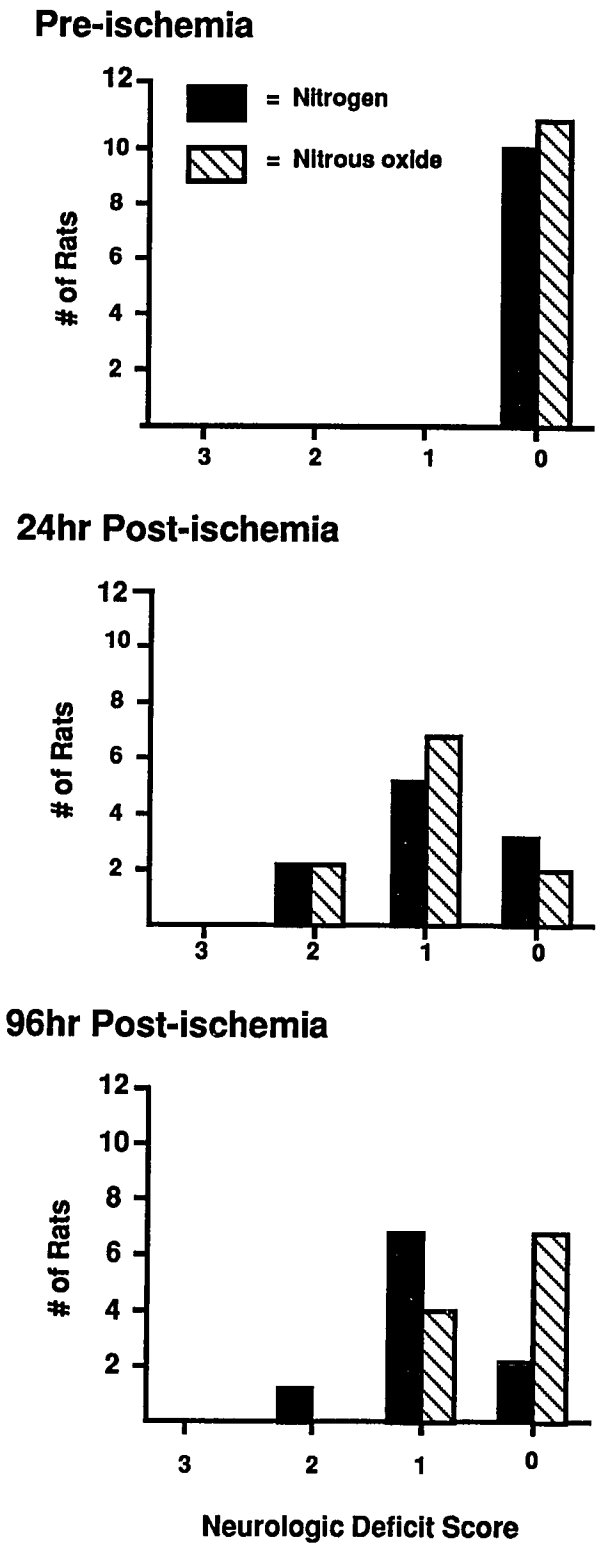

FIG. 4. Neurologic deficit scores according to the method of Bederson *et al.*[13] as a function of experimental interval and of whether nitrous oxide (70%) or nitrogen (70%) was present during middle cerebral artery occlusion in rats anesthetized with methohexital. A score of 0 indicates normal behavior. No differences between groups were observed at any interval. In both groups the scores were reduced at 24 h ($P < 0.01$), an effect that persisted through the 96-h postischemia observation interval ($P < 0.01$).

TABLE 2. Results From the Open-Field Test

	Preischemia	24 h Postischemia	96 h Postischemia
Nitrogen			
Squares entered	90 ± 31	28 ± 26*	33 ± 20*
Up	31 ± 10	4 ± 5*	7 ± 8*
Nitrous oxide			
Squares entered	97 ± 30	37 ± 27*	44 ± 32*
Up	33 ± 10	6 ± 12*	9 ± 16*

Values represent either the number of squares entered or the number of times the animal reared up on its hind legs within a 5-min observation period. Data presented as mean ± SD. *$P < 0.05$ comparing postischemic to preischemic values.

herein, that infarct volume is decreased in rats receiving deep methohexital as opposed to deep halothane or isoflurane anesthesia.[26] Interestingly, all of those studies finding barbiturates to be efficacious used intracranial vascular occlusion (*i.e.*, focal ischemia). In addition, it recently was reported that methohexital improves neurologic and histologic outcome in the rat (when compared to N_2O anesthesia) under conditions of moderate incomplete ischemia where EEG activity was allowed to persist.[28] Thus, it has generally been accepted that one critical determinant of barbiturate efficacy is whether the ischemic event is sufficiently severe to abolish cerebral function.[27]

Recently, this concept has been challenged with particular reference to the role of N_2O when co-administered with barbiturates during ischemic conditions. Although the potential for N_2O to increase CBF has been observed consistently in both humans and animals,[4-6,29] the effects of the same agent on CMR are less clear. Earlier studies in awake animals suggested that the effect of N_2O on CMR is minimal.[7,8] Later work observed a substantial increase in CMR_{O_2} when awake goats were administered 70% N_2O.[4] If N_2O is added to a background anesthetic, some studies have identified an increase in CMR while others have failed to find such an effect.[5,30-31] This is particularly relevant in the case of adding N_2O to a barbiturate anesthetic, wherein the effect of N_2O on CMR was found to be dependent on the dose of barbiturates administered. Specifically, the administration of N_2O to rats receiving a light barbiturate anesthetic (*i.e.*, active EEG) resulted in a 20–40% increase in CMR_{glu}, and the addition of N_2O to deep barbiturate anesthesia (*i.e.*, isoelectric EEG) left CMR_{glu} unchanged.[9] If N_2O causes an increase in CMR, it would seem reasonable to hypothesize that it would also attenuate the neuroprotective properties of barbiturates. There is some evidence for this concept.

TABLE 3. Physiologic Values for Methohexital Anesthetized Rats Undergoing CMRglu Determination in the Presence or Absence of Nitrous Oxide (70%) in the Inspiratory Gas Mixture

	Nitrogen (n = 5)	Nitrous Oxide (n = 5)
Pa_{O_2} (mmHg)	126 ± 16	121 ± 10
Pa_{CO_2} (mmHg)	38.3 ± 0.6	38.2 ± 1.3
Arterial *p*H	7.38 ± .08	7.41 ± .02
MAP (mmHg)	95 ± 5	93 ± 5
Plasma glucose (mg/dl)	94 ± 23	105 ± 21
Rectal temperature (° C)	37.1 ± 0.2	37.2 ± 0.2

There was not differences between groups.
Values are means ± SD.

Hartung and Cottrell employed a model that exposes mice to a hypoxic gas mixture and records the time until death (last perceptible breath).[10] If mice were injected intraperitoneally with thiopental before the hypoxic challenge, survival time was almost tripled from that observed in untreated animals. In contrast, if the hypoxic gas mixture contained 50% N_2O, the prolongation of survival time by thiopental was completely abolished. This observation led the authors to speculate that N_2O, presumably due to a metabolic stimulatory effect, might have also influenced outcome in other previous barbiturate protection studies. To evaluate that hypothesis, virtually all reported studies employing barbiturates were analyzed with respect to the presence or absence of N_2O during the ischemic/hypoxic insult, and a striking result was obtained. If N_2O was present, barbiturates failed to protect; however, if N_2O was absent, barbiturates did protect. Using chi-squared analysis, this observation was significant to $P < 0.01$.

There are several reasons to be skeptical of this conclusion. First, the mouse hypoxia model makes no distinction between the many physiologic variables that might influence time to death, including depth of anesthesia, blood glucose, blood pressure, respiratory drive (*i.e.*, respiratory depression), blood gases, and brain temperature. Second, many of the studies that were incorporated in the retrospective chi-squared analysis have been challenged because critical physiologic determinants were not controlled. We thus believed that a direct test of this hypothesis was indicated. Our current study found no difference in either neuropathologic or neurologic outcome as a function of the presence of N_2O in the respiratory gas mixture in rats undergoing focal ischemia during barbiturate anesthesia. Given the failure of N_2O to substantially alter CMR_{glu} under similar conditions, it can be concluded that at least in the scenario where near maximal metabolic suppression by barbiturates is invoked, N_2O will have no effect on outcome.

We believe that there should be some limitation to the interpretation of our results. It still remains unclear as to why the efficacy of barbiturates is dependent upon the insult being either focal or global in nature. Presumably, in the focal scenario, cells in the ischemic penumbra will retain some electrical activity and, hence, some metabolic activity that is suppressible by barbiturates. In our study, these cells would already have had their metabolic rates maximally depressed by the methohexital. Since N_2O failed to alter that metabolic rate, no difference in outcome would be expected. In contrast, as Sakabe *et al.* have demonstrated, N_2O will substantially increase CMR_{glu} under conditions of light barbiturate anesthesia.[9] Thus, the effect of N_2O on outcome from focal ischemia might be different if lighter levels of barbiturate anesthesia were used.

This hypothesis is supported to some extent by the recent work reported by Baughman *et al.* that investigates the protective efficacy of isoflurane in rats undergoing combined unilateral carotid artery occlusion and systemic hypotension (allowing persistence of some EEG activity).[32] If rats were administered 0.5 MAC of isoflurane during ischemia, neurologic outcome was better than that observed in rats receiving N_2O alone. In contrast, if the isoflurane was administered in combination with 70% N_2O, outcome became similar to that observed in rats receiving N_2O alone. While there is disagreement as to whether N_2O alters CMR in rats receiving 0.5 MAC isoflurane,[30] that study represents the only outcome study evaluating the effects of N_2O when co-administered with light levels of a metabolic depressant anesthetic. In that case, the ability of a depressant drug to act as a cerebral protectant appeared to be altered by the co-administration of N_2O.

In conclusion, we observed no difference in either pathologic or neurologic outcome from 2 h of temporary focal ischemia in rats receiving a deep barbiturate anesthetic whether N_2O was present in the respiratory gas mixture. In addition, CMR_{glu} was not different between

TABLE 4. CMR_{glu} Values* ($\mu mol \cdot 100\ g^{-1} \cdot min^{-1}$) in Methohexital Anesthetized Rats as a Function of the Presence or Absence of 70% Nitrous Oxide in the Respiratory Gas Mixture

	Nitrogen (n = 5)	Nitrous Oxide (n = 5)
Caudoputamen	23 ± 3	20 ± 3
Corpus callosum	14 ± 4	12 ± 3
Neocortex	22 ± 3	20 ± 3
Septal nucleus	23 ± 2	21 ± 4
Hypothalamus	22 ± 1	20 ± 2
Thalamus	30 ± 2	30 ± 5
Hippocampus	25 ± 4	22 ± 4
Lateral geniculate	28 ± 8	25 ± 6
Substantia nigra	31 ± 4	33 ± 3

* Means ± SD.

the two groups. Our results leave open the theoretical possibility that cerebral protection could be attenuated by N_2O during different anesthetic conditions. However, our results are consistent with the classical interpretation of barbiturate mechanisms of action in focal ischemia and speak strongly against the hypothesis that the protective efficacy of deep barbiturate anesthesia can be predicted by whether N_2O was present or absent.

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