

Effects of Nitrous Oxide on the Cerebrovascular Tone, Oxygen Metabolism, and Electroencephalogram of the Isolated Perfused Canine Brain

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Nitrous oxide has been reported to act both as a stimulant and as a depressant of cerebral oxygen metabolism (CMR_{O_2}) and blood flow under a variety of experimental conditions in the intact animal. The isolated brain preparation is advantageous because it permits direct measurement of blood flow and allows the study of drug effects without interference from other organ systems or drugs. In this study, six isolated perfused canine brain preparations were used to compare the CMR_{O_2} , cerebral vascular resistance (CVR), and the EEG of brains perfused with normocapnic, normoxic blood equilibrated with either 70% N_2O or 70% N_2 . There was no significant change in CMR_{O_2} . Cerebral vascular resistance fell [$16.4\% \pm 3.4\%$ SEM ($P < 0.015$)] during exposure to N_2O . The EEG pattern was reduced in amplitude, but showed an increase in both low-voltage β activity (14–40 Hz), and 3–5 Hz activity. In the isolated brain, N_2O reduces cerebral vascular tone while exhibiting no effect on cerebral oxygen metabolism. (Key words: Anesthetics, gases; nitrous oxide. Brain: blood flow; electroencephalography; oxygen consumption.)

DURING THE PAST TWO DECADES the effects of nitrous oxide on cerebral blood flow and metabolic activity were discussed in a number of reports; however, results of those studies were often contradictory.^{1–7} Wollman *et al.* observed a 23% decrease in cerebral metabolic rate for oxygen (CMR_{O_2}) in humans.¹ Theye and Michenfelder, in a study with dogs, found an 11% increase in CMR_{O_2} ,² while Carlsson *et al.*, using rats, observed no change in CMR_{O_2} .³ Similar variability was seen in studies of cerebral blood flow and cerebral vascular tone. The dissimilarity in results probably can be accounted for by differences in background anesthetic drugs and in measurement techniques.

In an attempt to overcome some of the limitations of other studies, we compared the metabolic and physiologic effects of 70% nitrous oxide with those of 70% nitrogen in a normoxic, normocapnic system used to perfuse the isolated canine brain. In these experiments no other organs were present, no other drugs were administered during the studies, and flow was measured directly.

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Received from the Departments of Anesthesiology, Neurosurgery, and Physiology, University of Wisconsin Center for Health Sciences, Madison, Wisconsin. Accepted for publication May 20, 1982. Supported by grant NS05961 from the National Institute of Neurological Diseases, Communicative Disorders and Stroke.

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Materials and Methods

The brains of six mongrel dogs (8–15 kg) were surgically isolated as described in a previous communication.⁸ The procedure conformed to the American Physiological Society standards for treatment of experimental animals. Intubation was facilitated with intravenous succinylcholine. Anesthesia was induced and maintained with a halothane–oxygen mixture, and the dog was ventilated mechanically. A tracheostomy was performed, and a cutdown was made for placement of the femoral artery cannula used to monitor blood pressure. The soft tissue surrounding the skull was excised surgically by electrocautery. The carotid arteries were identified, and all branches except the internal carotid and the anastomotic branch of the internal maxillary artery were ligated. The mandible, zygomatic arches, snout, and eyes were removed. Heparin was administered and T-tubes were then placed in the common carotid arteries at the level of the cranial thyroid artery. No other agents were administered during the preparation. A laminectomy was performed at the level of the second cervical vertebra, and the spinal cord was ligated and transected. At this time, mechanical perfusion was begun and the carotid arteries were severed and ligated proximal to the T-tubes. Venous drainage was accomplished via a threaded Luer connector fixed in a small hole drilled into the confluence of sinuses. The vertebral arteries were identified and ligated at the level of C2. The validity of the preparation was assessed in terms of oxygen utilization and the EEG pattern.^{9,10}

The perfusion apparatus consisted of two separate roller pump-membrane oxygenator systems that were interconnected by means of a rotary valve which permitted rapid change from one perfusion system to the other without loss of flow or perfusion pressure. The systems were primed with blood obtained from an immunologically compatible donor dog. This blood was conditioned¹¹ and diluted to a hematocrit of 30%.

Blood pH, P_{O_2} , and P_{CO_2} were measured using an IL 313 Blood Gas Analyzer (Instrumentation Laboratory Inc.) with suitably calibrated electrodes. Blood oxygen content was determined with a Lex- O_2 -Con (Lexington Instruments Corp.), which was calibrated with a VanSlyke blood-gas apparatus. Plasma glucose was assayed by a glucose-oxidase method (Beckman Glucose

TABLE 1. Results After Fifteen Minutes of Perfusion with 70% Nitrous Oxide

	DOG						Average
	1014	1018	1019	1021	1024	1026	
CMR _{O₂} (Cont)	5.51	4.84	5.82	5.33	5.53	6.16	5.53 ± 0.20 SEM
CMR _{O₂} (N ₂ O)	5.33	5.32	5.94	5.09	5.89	5.87	5.57 ± 0.16 SEM
CMR _{O₂} (Post)		4.67	5.86	5.04	5.17	5.58	5.26 ± 0.23 SEM
CMR _{O₂} Change	-3.2%	+9.8%	+2.1%	-3.3%	+6.6%	-4.7%	1.2% ± 2.7 SEM
PV _{O₂} (Cont)	36	36	33	31	34	30	33.3 ± 1.1 SEM
PV _{O₂} (N ₂ O)	36	31	34	31	34	30	32.6 ± 1.1 SEM
PV _{O₂} (Post)	36	34	34	29	35	30	33.0 ± 1.3 SEM
PV _{O₂} Change	0	-5	+1	0	0	0	-0.7 ± 0.9 SEM
CVR (Cont)	4.47	3.72	4.26	3.36	1.95	2.23	3.33 ± 0.46 SEM
CVR (N ₂ O)	3.14	3.13	3.86	2.88	1.66	2.01	2.78 ± 0.36 SEM
CVR (Post)	3.37	3.68	4.91	3.58	2.07	2.20	3.30 ± 0.47 SEM
CVR Change	-29.8%	-16.0%	-9.5%	-14.2%	-19.3%	-9.7%	16.4% ± 3.4% SEM

Cont = Control; N₂O = after 15 min of 70% nitrous oxide; Post = after 15 min of reperfusion with nitrogen and oxygen.

Units: CMR_{O₂}, ml · 100 g⁻¹ · min⁻¹; PV_{O₂}, mmHg; and CVR, mmHg/(ml · 100 g⁻¹ · min⁻¹).

Analyzer). Perfusion pressure was recorded continuously on a Grass Instruments Polygraph (Model 78D) utilizing Statham P-23AA transducers. A 6-lead EEG was recorded continuously on the Grass Polygraph, and magnetically recorded using a Wolff Industries 4-channel FM cassette tape recorder. Cup electrodes filled with electrode paste were placed directly onto the calvarium and held in place with bone wax. Frontoparietal, parietooccipital, and occipitofrontal leads on both the right and left sides were recorded. Fourier transform frequency-amplitude spectrum analysis was performed on the taped data by means of a program developed for the PDP 11/34 computer.‡ Venous blood was analyzed for halothane by the method of Cowles *et al.*¹²

Following isolation, the brain was perfused for 1 h with a normoxic, normocapnic mixture of blood equilibrated with 70% N₂, 25% O₂, and 5% CO₂. During this time most of the small amount of halothane remaining in the system was eliminated from the oxygenator, and the cerebral consumption of oxygen reached values normally observed in unanesthetized dogs. The venous halothane concentration after 45 min of perfusion was 20.2 ppm. Control samples of arterial and venous blood were analyzed for pH, P_{O₂}, P_{CO₂}, oxygen content, and glucose concentration. The arterial pH was maintained between 7.38 and 7.42, the Pa_{O₂} between 170 and 190 mmHg, the Pa_{CO₂} between 38 and 42 mmHg, and the arterial glucose between 80 and 100 mg/dl. Perfusion pressure and the EEG were recorded continuously throughout the experiment. Although cerebral blood flow was maintained between 55 and 70

ml · 100 g⁻¹ · min⁻¹, it remained constant within the individual studies. Blood flow was measured directly by collecting venous blood from the confluence of sinuses for 1 min in a graduated cylinder.

After the control samples were taken, perfusion was switched to a second pump-oxygenator system which was primed with blood equilibrated with a normocapnic, normoxic gas mixture containing 70% N₂O. Blood samples were taken at 1, 5, 10, and 15 min for blood-gas, glucose, and oxygen analysis. Blood flow also was determined at these same intervals. Following 15 min of perfusion with blood containing N₂O, perfusion was returned to the control oxygenator for one hour. Blood samples were obtained and flows measured at 5, 15, 30, and 60 min following exposure to N₂O.

Although the system contained two bubble traps, preliminary experiments indicated that air microemboli containing N₂O reached the brain after 25 to 30 min of perfusion with experimental blood. Because this caused a marked increase in CVR and decrease in CMR_{O₂}, perfusion with N₂O was limited to 15 min. There were no problems with air emboli during perfusion with control blood. At the conclusion of the experiment, the brain was removed from the calvarium and weighed.

The cerebral metabolic rate for oxygen (CMR_{O₂}) in ml · 100 g⁻¹ · min⁻¹ is equal to (A - V) · F/W where (A - V) is the arteriovenous oxygen content difference in ml O₂/100 ml blood, F is blood flow in ml/min, and W is brain weight in grams. Cerebral vascular resistance (CVR) in mmHg/(ml · 100 g⁻¹ · min⁻¹) is the perfusion pressure in mmHg, divided by the blood flow in ml/min per 100 g of brain weight. Each dog served as its own control.

The mean and standard error of the mean (SEM) for

‡ The program was developed by F. Sasse, M.D. and I. Rampil, M.S., of the Department of Anesthesiology, University of Wisconsin, Madison.

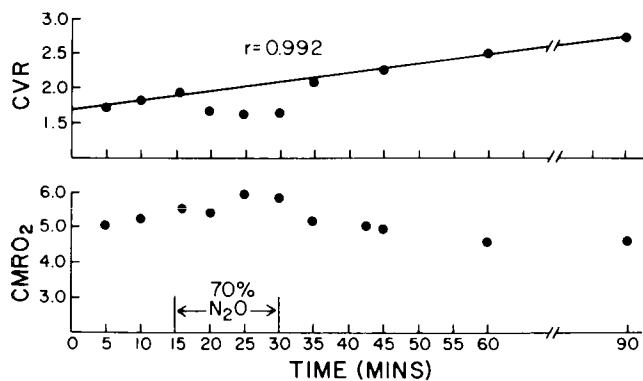


FIG. 1. Changes in CVR [mmHg/(ml·100 g⁻¹·min⁻¹)] and CMRO₂ (ml·100 g⁻¹·min⁻¹) vs. time in minutes for dog 1018. Note the gradual but sustained decrease in CVR and the rapid recovery following removal of N₂O.

the changes in CMRO₂ and CVR were calculated and their significance assessed using a single tailed Student's *t* test for paired data.

Results

Compared to control values, the change in CMRO₂ during N₂O perfusion was variable, decreasing in three

brains, and increasing in the remaining three (table 1). The change approached 10% in only one brain and averaged an insignificant 1.2% ± 2.7% SEM (*p* = 0.32). No change in venous P_{O₂} was observed during N₂O administration.

The cerebral vascular resistance decreased in all dogs during perfusion with blood containing N₂O (table 1) falling an average of 16.4% ± 3.4% SEM after 15 min of perfusion with experimental blood. This change was significant (*P* < 0.015). The fall in resistance was gradual, and continued throughout the 15 min of exposure to N₂O (fig. 1).

The pattern of the 6-lead EEG exhibited an increase in both low-voltage β (14–40 Hz), and 3–5 Hz activity during perfusion with blood equilibrated with N₂O. Figure 2 is a representative frequency amplitude spectrum. From this figure, it can be seen that changes occur quickly following the administration of N₂O; however, the EEG reverts rapidly to a control pattern following removal of N₂O.

Discussion

The diverse results observed by other investigators with respect to the effects of N₂O on cerebral vascular

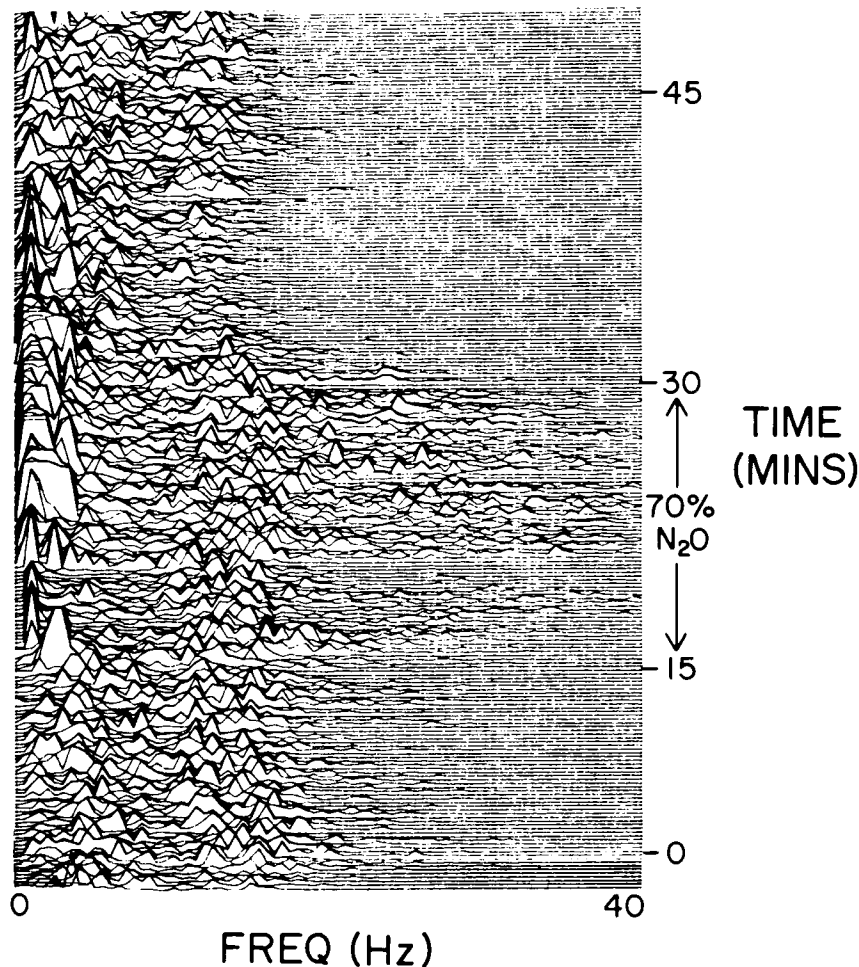


FIG. 2. Frequency-amplitude spectrum analysis for a continuously recorded right occipitofrontal lead of dog 1021. Note the rapid onset of change and the recovery following removal of N₂O.

tone and oxygen metabolism are probably the result of factors unrelated to N₂O. In a recent editorial, Siesjö commented on several problems involved in investigations of cerebral blood flow and metabolism.¹³ Among them are: 1) the effects of anesthetics, 2) drug interactions, 3) species differences, 4) problems with flow measurements, and 5) the variety of techniques used to measure CMR_{O₂}.

Many previous studies involved either premedication or simultaneous use of other drugs such as thiopental or halothane. Effects of N₂O and other drugs are not necessarily additive.¹⁴ Flows were measured by indirect techniques except in the Theye and Michenfelder study.² Also, intact animals were used. The Carlsson *et al.* investigation provides a classic example of the influence that other organs and drugs may have on cerebral metabolic and flow studies.³

The isolated brain preparation overcomes several of these problems. There are no effects from anesthetics other than the one under investigation, there are no other drugs involved, flow is measured directly from only the vascular bed under study, and blood oxygen content is measured using a fuel cell.

The major disadvantages of the preparation include a gradual rise in CVR, regional blood flow is not identical with *in vivo* values, flow is not physiologic, and the cerebrovascular bed is denervated. The gradual rise in CVR is probably caused by circulating vasoactive substances such as 5-hydroxytryptamine, ADH, kinins, and catecholamines. Additionally, microemboli occasionally escape the filters and bubble traps. Studies are done only if metabolic and electrophysiologic criteria of viability are met.¹⁰ This continuous rise in CVR makes it difficult to quantitate the observed changes in a simple way, but trends and magnitudes can be measured, particularly if they are seen consistently in a number of experiments. Regional blood flow to the hindbrain areas is reduced.¹⁵ This could have an effect on the EEG since some areas of the reticular activating system are not normally perfused.

The results indicate that N₂O had a significant vasodilating effect on the cerebral vessels. In general, decreases in vascular tone caused by drugs may be mediated by adenosine, direct β stimulation, direct action on smooth muscle cells, or inhibition of endogenous norepinephrine.¹⁶⁻¹⁸ § Several of these mechanisms may be operant here. Nitrous oxide has been shown to decrease the contractile force of the dog and cat myocardium.^{19,20} *In vivo*, N₂O stimulates sympathetic activity, and increases circulating norepinephrine levels.^{21,22} Thus, changes in myocardial contractility and in circulating catecholamine levels may be responsible for

some of the changes in CBF and vascular tone observed in the intact animal.

Although the cranial cervical ganglion was ablated and the extracranial vessels denervated, the neuromuscular junctions of the intracranial vessels were intact and maintained their norepinephrine stores in this preparation. Studies done in this laboratory have demonstrated adrenergically mediated changes in vascular tone of cerebral vessels of the isolated brain preparation, as well as changes mediated by drugs acting through other mechanisms.²³⁻²⁵ Thus, vascular tone could have been influenced by endogenous norepinephrine stored in these nerve endings, as well as by circulating vasoactive substances such as catecholamines, prostaglandins, ADH, kinins, and 5-hydroxytryptamine.¹⁶ Musch *et al.* reported that N₂O causes a metabolic acidosis in the extracellular fluid environment of the brain.²⁶ This could lead to vasodilation by direct action of the hydrogen ion on arteriolar smooth muscle.²⁷ Adenosine has also been proposed to have a significant role in the regulation of cerebrovascular tone. § The vasodilation of the cerebral vessels by N₂O seen in this preparation is similar to the effects of most anesthetic agents on the microvasculature as was discussed in reviews by Longnecker and Harris²⁸ and Altura *et al.*,²⁹ and in a report on N₂O by Longnecker.³⁰

There was no significant effect of N₂O on cerebral oxygen metabolism. During the preparation the brain is deafferented. Consequently, it receives no stimuli during the experiment. Although it seems likely that the brain is in a sleep-like state during perfusion with control blood,³¹ the CMR_{O₂} values measured during this period are similar to those observed in the intact, awake dog.² The control EEG pattern was comparable to those observed in the intact animal, while the patterns during N₂O perfusion and recovery parallel the effects seen clinically.³²

In an earlier study,³³ we demonstrated that administration of sodium pentobarbital lowered the cerebral metabolic rate for oxygen in the isolated dog brain. Hostetler *et al.*³⁴ and Watanabe *et al.*³⁵ obtained similar results in the isolated brains of the monkey and cat, respectively. Thus, isolated brains behave in much the same manner as the intact brain where it is also wise to avoid use of barbiturates and other anesthetic agents when studying metabolism.

In this study, we have shown that N₂O administered at a 70% concentration is a significant dilator of cerebral vessels, and has an insignificant effect on CMR_{O₂}. Additional experiments with other agents are in progress. Further studies on the effects of N₂O on vascular tone following adrenergic blockade or pre-experimental ablation of the cranial cervical ganglion would be of interest. These results should also offer comfort to those investigators who use animals anesthetized with 70% N₂O as controls during studies of cerebral metabolism.

§ Winn HR, Rubio GR, Berne RM: The role of adenosine in the regulation of cerebral blood flow. *Journal of Cerebral Blood Flow and Metabolism* 1:239-244, 1981.

The authors thank Ms. Jan Yanushka for her technical assistance, David C. Flemming, F.F.A.R.C.S., for his help in editing the manuscript, and Ohio Medical Anesthetics who generously provided the blood halothane analysis.

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