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Nitrous Oxide Markedly Increases Cerebral Cortical Metabolic Rate and Blood Flow in the Goat

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Studies to date on the influence of N_2O on cerebral blood flow (CBF) and metabolism in dogs, rodents, and humans have produced conflicting results. In the present study the authors have employed techniques in the awake, freely breathing nonstressed goat that allowed the authors to 1) serially obtain rapidly frozen cerebral cortical biopsy specimens (for labile metabolite assay) and 2) measure changes in cerebral O_2 metabolism ($CMRO_2$) and total and regional CBF (rCBF). Thus, with each animal utilized as its own control, the authors studied N_2O effects on the above variables. Two determinations of the effects of 1 h of N_2O (70% via a mask) on these variables were performed on each animal. Following introduction of N_2O, Pa_{CO_2} and arterial blood pressure did not change, but arterial epinephrine levels declined over the 60-min period. Total CBF increased in the first 5 min of N_2O exposure, reached a maximum of 165% control at 15 min, and then decreased to 143% control at 60 min. rCBF evaluations showed that much of this CBF increase was confined to cerebral cortical structures (188–246% control at 60 min). Over the same period cortical $CMRO_2$ increased to 170% of control. No appreciable changes in the levels of high-energy phosphates or glycolytic intermediates were found at 60 min of N_2O. The authors attribute the described changes solely to the presence of N_2O and not to sympathoadrenal influences, altered ventilation, or anything related to the experimental preparation, and they conclude that N_2O (at least in goats) is associated with a marked cerebral cortical "activation." (Key words: Anesthetics, gases: nitrous oxide. Brain: blood flow; metabolism.)

A TROUBLESOME PROBLEM confronting investigators in studies involving assessment of cerebral metabolic function, metabolite levels, and hemodynamics is in defining the control situation. The profound effects of most anesthetic agents (when given in anesthetic concentrations) on the above variables precludes their use in investigations where experimental influences on these variables are being evaluated (for example, see Chapman *et al.*,[1] Abdul-Rahman *et al.*,[2] and Michenfelder and Theye[3]).

Studies to date on the influence of nitrous oxide on CBF and cerebral metabolism have produced conflicting results. A number of studies have variously reported that exposure to N_2O (60–70%) is associated with an increase in cerebral blood flow (CBF),[4–7] an elevated cerebral metabolic rate for oxygen ($CMRO_2$),[4–7] and glucose (CMRgl),[8] and a slight change in the pattern of glycolytic metabolites.[9] Conversely, others have found only minimal influences of N_2O on these variables.[10–15] This lack of agreement among studies may be due, in part, to differences in experimental preparations that have included (either together or separately) paralysis and artificial ventilation, stress-inducing mechanical restraint, additional anesthetics, and extensive acute surgical preparation. Clearly, this could complicate defining the "control" situation.

In the present study, we have employed techniques in the spontaneously breathing goat where the influences of stress have been minimized. These techniques allowed us to obtain rapidly frozen serial samples of cortical tissue (for metabolite analysis) and do multiple determinations of CBF (local and whole brain) and cerebral metabolic rates in awake and N_2O anesthetized goats. A major advantage of this approach is that each animal can be used as its own control.

Materials and Methods

Nine female goats (25–35 kg) were used in the study. Two determinations of the effects of 1 h of nitrous oxide (70%/30% O_2, mask delivered) on labile cerebral cortical metabolite levels (high-energy phosphates and selected glycolytic and TCA cycle intermediates), metabolic rate ($CMRO_2$), and CBF were performed on eight animals. In one goat, three evaluations were made. For each animal, we allowed 2 to 3 days between evaluations. All goats were surgically prepared 6–8 days prior to study with an acrylic "cranial window" for use in brain biopsy (see below) and femoral arterial, femoral venous, and sagittal sinus catheters. Seven goats were fitted with a left atrial catheter for injection of microspheres and regional CBF (rCBF) determinations. In addition, an electromagnetic flow probe was implanted on the internal maxillary artery in *all* animals, following elimination of extracerebral flow, for continuous *estimation* of total CBF[16,17] (also see below).

In the standard experimental protocol, goats were placed in a stanchion, which limited head movement and provided some body support. An airtight, rubber-cuffed mask was placed on the goat. During the awake control condition, 70% N_2/30% O_2 was delivered to the mask. Following a 1.5–2.0-h control period, 70% N_2O (balance

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O_2) was introduced and maintained for a period of 1 h. The N_2O was switched off, the control conditions were reimposed, and the goat was monitored an additional 1.0–1.5 h. For rCBF evaluations, single control microsphere injections were made either 20–30 min prior to introduction of N_2O or at 60–90 min following cessation of N_2O. During N_2O exposure, microspheres were injected at the end of the 1-h period. Control cortical tissue biopsy specimens (for metabolite assay) were taken either immediately preceding N_2O introduction or at 60–90 min following reimposition of control conditions. During N_2O, biopsy specimens were taken at the end of the one hour period. In experiments involving rCBF measurements (n = 14), biopsy specimens were taken following completion of each microsphere injection. Further details on the procedure for rCBF evaluation and cerebral cortical biopsy are presented below. Relative total CBF, mean arterial blood pressure (MABP), and rectal temperature were monitored continuously. Rectal temperature remained at 39–40° C throughout. Arterial blood was sampled at 30-min intervals and analyzed for Pa_{O_2}, Pa_{CO_2}, and plasma glucose. Additional arterial blood was taken during pre-N_2O control conditions (three to four samples) and at 30 and 60 min of N_2O exposure for analysis of epinephrine and norepinephrine levels.

Cranial Window Preparation and Biopsy Procedure

The cranial windows are made of transparent acrylic. The basic unit consists of an outer ring and a solid, threaded central plug. The plug can be unscrewed for access to the cortical surface (dura excised). The unit was implanted under anesthesia (halothane or enflurane) and aseptic conditions. The specific surgical procedure is outlined, in detail, in a previous report.[18]

A suction freezing apparatus was used for cortical tissue biopsy. The design is presented elsewhere.[18] The biopsy system allowed us to serially obtain rapidly frozen (<0.5 s) 100–250-mg tissue samples from the parietal cortex. One to two samples were obtained during control and during N_2O exposure. For repeat biopsies, we allowed 5–10 min between samples. Repeat samples were analyzed separately but grouped together as one value following analysis. Therefore, on a given day, a single control metabolite profile was paired with a single N_2O metabolite profile. No significant intraanimal variation in the metabolite response was observed when comparing evaluations. Therefore, the reported metabolite values (and changes during N_2O) are based on 19 paired observations. All samples were stored at −80° C until analysis. No appreciable bleeding has been observed yet following sampling. Furthermore, no signs of sampling (or immobilization) stress were evident with present procedures. This was demonstrated by the absence of any significant variations in arterial catecholamines, Pa_{CO_2}, or blood pressure during the entire period of evaluation including biopsies (see Pelligrino *et al.*[18]). Following biopsy, no apparent motor or behavioral abnormalities were observed in any animal.

Regional CBF Measurements

Four determinations of regional CBF (two on each day of evaluation) were made in each animal using ^{57}Co, ^{113}Sn, ^{85}Sr, and ^{46}Sc labelled 15-μm microspheres (New England Nuclear). The injection sequence (in the order of isotopes given above) and pairing of results was either control-N_2O (day 1), control-N_2O (day 2) (four goats), or N_2O-control (day 1), N_2O-control (day 2) (three goats). The sequence was varied in order to check whether the order of isotope injection might bias the results in any way. A nearly identical local CBF response to N_2O was found when comparing the two injection sequences (*i.e.*, no independent "isotope effect"). Furthermore, no difference in the response to N_2O was seen when comparing the two observations in each animal (*i.e.*, no signs of response enhancement or tolerance). Thus, local CBF changes are based on 14 paired observations. Microspheres were suspended in isotonic saline using 0.05% Tween-80. Five milliliters of the suspension, containing about 2.5×10^6 microspheres, were injected into the left atrium over 15 s and flushed in with saline. Just prior to injection, pump withdrawal of blood, at 10 $ml \cdot min^{-1}$, from the femoral arterial catheter was initiated. This was continued then for 2 min following injection. The total blood counts at the above withdrawal rate were used as the reference flow for subsequent calculations. Following the final isotope injection (and brain biopsy), the goat was killed via injection of sodium pentobarbital followed by KCl, and the brain was removed and placed in formalin overnight. Tissue, from 10 regions on both sides of the brain, was dissected out, weighed, and counted. Approximately 0.4–2.0 g of tissue were taken from the frontal cortex, occipital cortex, "non window" parietal cortex, cerebellum, pons, thalamus, hypothalamus, hippocampus, caudate nucleus, and the tissue under the window (parietal) and in the equivalent parietal region from the contralateral cortex.

Cerebral Metabolic Rate

$CMRO_2$ was estimated by multiplying the femoral arterial and sagittal venous differences for O_2 content by the average *cortical* (*i.e.*, parietal + frontal) blood flow. Several paired arterial and venous samples were drawn prior to and following microsphere injection. The A-V differences then were derived by averaging the values obtained from these samples.

TABLE 1. Arterial Blood Measurements

	Pa_{O_2} (mmHg)	Pa_{CO_2} (mmHg)	pH_a	$[HCO_3^-]$ (mmol · l^{-1})	Glucose (mg · dl^{-1})	MABP (mmHg)	Epinephrine (pg · ml^{-1})	Norepinephrine (pg · ml^{-1})
Awake Control	156 ± 12	39.9 ± 1.3	7.384 ± 0.012	23.0 ± 0.7	93 ± 4	112 ± 5	605 ± 131	471 ± 71
70% N_2O								
30 min	144 ± 8	40.2 ± 1.2	7.364 ± 0.010	22.7 ± 0.7	96 ± 5	118 ± 4	349* ± 63	535 ± 55
60 min	152 ± 16	39.8 ± 1.3	7.367 ± 0.009	22.4 ± 0.7	98 ± 5	107 ± 9	375 ± 99	642 ± 86

Number of observations = 19.
All values are x ± SEM.

* $P < 0.05$.

TOTAL CBF MEASUREMENTS

CBF was measured continuously using a Statham #2200 blood flow measuring system with electrical zero calibration. With this system, one measures flow through the internal maxillary artery on one side only and therefore must assume that the flow one measures does not include flow to the opposite hemisphere. Furthermore, little or no extracerebral flow should be included in the measurement. Some crossover and extracerebral flow does exist with this procedure and accounts for about 15% of the measured flow (see Miletich *et al.*[17]). Total CBF was obtained, therefore, by multiplying the measured flow by two, making a 15% correction, and then dividing by brain weight. For evaluation of results on each day of experimentation, CBF values at 5, 15, 30, and 60 min of N_2O and at 10 min post-N_2O were compared with the average control CBF obtained during the period preceding N_2O exposure *and* to each other. No appreciable systematic differences could be found when comparing results from the first day to subsequent days, thus (as above) evaluation of results is based on 19 observations.

ANALYSES

Arterial blood PO_2, P_{CO_2} and pH (with temperature corrections) were measured in an IL 1303 blood–gas analyzer. $[HCO_3^-]$ values were calculated by the IL 1303 utilizing appropriate pK′ and solubility factors for CO_2. The O_2 content of arterial and sagittal venous blood was determined with an IL-282 CO-oximeter. Plasma epinephrine and norepinephrine levels were determined using a radioenzymatic method as described by Passon and Peuler.[19] The glucose level in arterial plasma was determined with a Beckman glucose analyzer-2. Brain tissue and blood sample radioactivities in microsphere injected animals were evaluated using a Nuclear Chicago 1085 gamma counter and Nuclear Data 600 multichannel analyzer. Regional CBF values were calculated according to Heymann *et al.*[20]

The frozen tissue samples were extracted at −20° C in HCl/methanol followed by precipitation of proteins in 0.3 M $HClO_4$ at 0° C. Brain tissue metabolites in the neutralized deproteinized extracts then were analyzed using enzymatic fluorometric techniques according to Lowry and Passonneau.[21] Details of the extraction procedure and the analytical conditions used have been outlined previously.[22–25] In most cases results were analyzed using a paired Student's *t* test (based on differences between test and control). Total CBF results and blood data (and intraanimal comparisons for all other measurements) were analyzed employing a repeated measures analysis of variance, using a Bonferroni *t* test for multiple pairwise comparisons.

Results

During the first several minutes following introduction of N_2O into the inspiratory gas mixture, no changes in the goats' behavior could be detected. The animals remained standing and quiescent. After approximately 10–15 min and for the remainder of the N_2O exposure period, the goats appeared, in most cases, lethargic with eyelids closed. Furthermore, these same animals were not able to stand without the aid of a sling supporting the chest and abdomen. No animal showed any signs of excitation during the N_2O exposure period. Arterial blood PO_2, P_{CO_2}, pH, $[HCO_3^-]$, and mean arterial blood pressure (MABP), together with arterial levels of glucose, epinephrine, and norepinephrine in awake controls and at 30 and 60 min of 70% N_2O exposure, are given in table 1. These variables remained relatively constant during the N_2O exposure period, with the exception that epinephrine levels were reduced from control. However, only the 30-min value (57% control) was found to be significantly changed ($P < 0.05$).

CEREBRAL BLOOD FLOW

The total (whole brain) CBF was increased rapidly from the control value of 60 ± 5 ml · 100 g^{-1} · min^{-1} (fig. 1). Most of this change occurred over the first 5 min (*i.e.*, to 151% of control), reached a maximum value of 165% of awake control at 15 min, and diminished gradually to

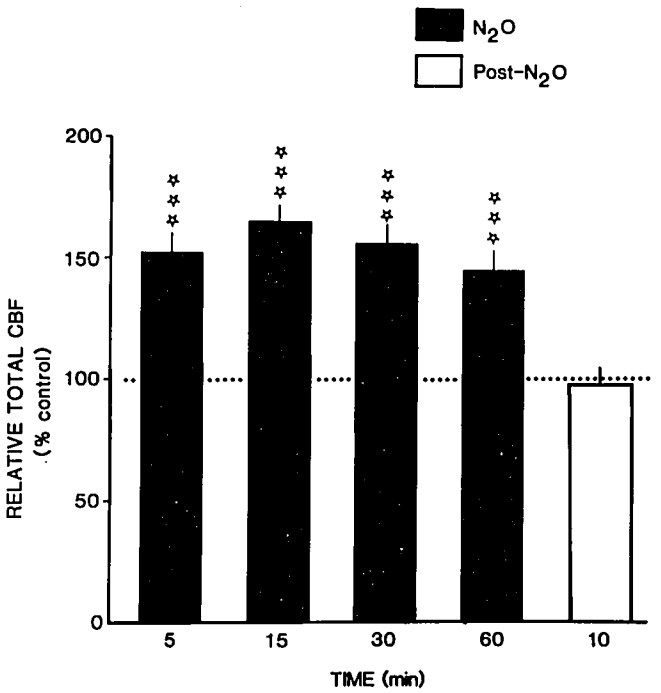

FIG. 1. Total CBF changes over 60 min of N_2O exposure and at 10 min following reintroduction of control inspiratory gases (19 observations). Each bar represents the mean (±SEM) value recorded at the time indicated expressed as a per cent of the control value of 60 ± 5 ml·100 g^{-1}·min^{-1}. The three stars indicate significance at the $P < 0.001$ level.

143% of control at 60 min. When comparing CBF values during N_2O exposure, the value at 60 min was found to be significantly less ($P < 0.05$) than the 15-min value. CBF was found to return to normal by 10 min following reimposition of control conditions. Regional CBF (rCBF) changes at 60 min of N_2O exposure are shown in figure 2. All values and changes represent an average for each region of both right and left sides of the brain. This was done because no significant right to left variations were found. As can be seen, much of the CBF increase was confined to cortical tissue. The highly significant (expressed as a percentage of the control value) rCBF changes in the four cortical regions shown ranged from 188% (window area–parietal cortex) to 246% (frontal cortex). In deep structures, rCBF changes were of a much smaller magnitude. These changes ranged from not significantly different from control (thalamus, hypothalamus) to modest but significant increases in hippocampus (141%), pons (160%), caudate nucleus (119%) and cerebellum (134%).

CEREBRAL METABOLIC RATE

A highly significant increase in $CMRO_2$ (170% of control) was found at 60 min of N_2O exposure (table 2).

METABOLITE LEVELS

No changes in the brain tissue levels of phosphocreatine (PCr), ATP, ADP, AMP, glucose-6-phosphate, fructose-

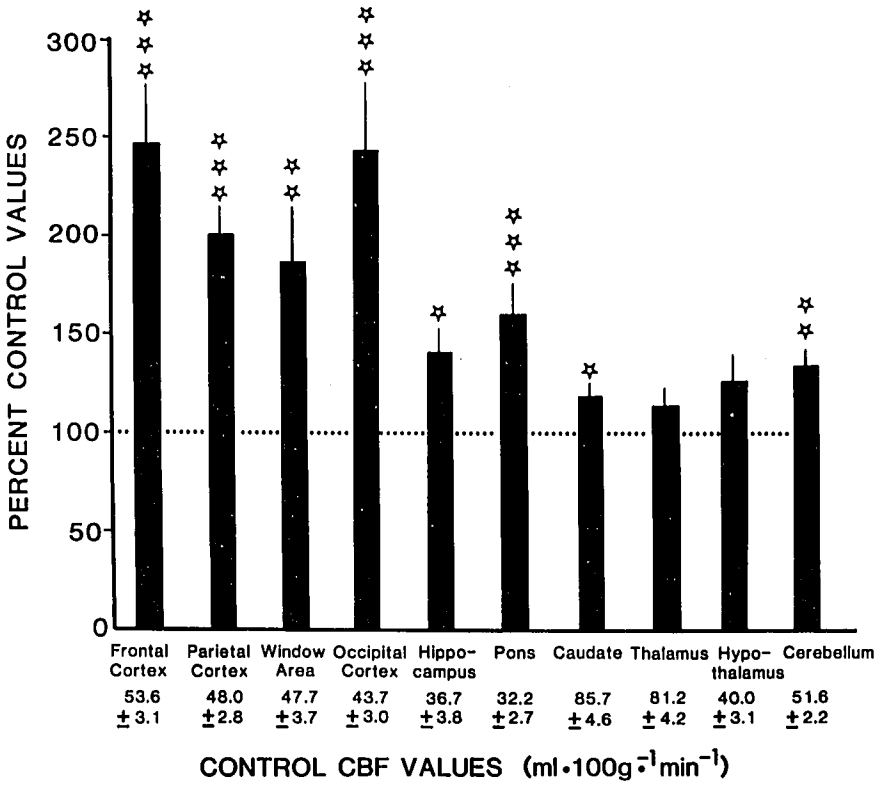

FIG. 2. Regional CBF (rCBF) changes at 60 min of N_2O (14 paired observations). Bar format is the same as figure 1. Also shown are the control rCBF values (in ml·100 g^{-1}·min^{-1} ± SEM). One, two, or three stars indicate, respectively, significance at the $P < 0.05$, $P < 0.01$, $P < 0.001$ level.

6-phosphate lactate, or pyruvate were found during N_2O exposure (fig. 3). The only measured metabolite affected by N_2O was citrate (130% control, $P < 0.05$).

Discussion

Present results clearly demonstrate that in freely ventilating, nonstressed goats, 70% N_2O rapidly increases CBF, primarily in cortical structures, and enhances cerebral cortical oxygen utilization. Furthermore, 60 min N_2O exposure was shown to *not* be associated with *appreciable* changes in the pattern of metabolites in the glycolytic pathway or in the levels of labile phosphates. These results probably can be attributed to a direct effect of N_2O on cerebral metabolism and blood flow and not to altered ventilation or factors related to the basic experimental preparation. This was evidenced by a lack of variation in arterial P_{CO_2} in the present study *and* by the previous demonstration[18] that the present goat model is not associated with any appreciable signs of stress, acute or chronic alterations in CBF and its distribution, or time-related variations in the tissue metabolite pattern.

Since it has been reported that N_2O can increase sympathetic activity,[26–28] an action that may originate at supra-pontine levels,[28] one must consider the possibility that the increased cerebral metabolism and flow is the result of sympathoadrenal activation. However, we found no sign of any N_2O-induced *increase* in arterial blood catecholamines, glucose, or pressure. These measurements certainly are not as accurate as measurement of sympathetic nerve discharge, but it should be noted that direct artificial stimulation of preganglionic sympathetic nerves (the same nerves where increased activity has been recorded during N_2O[27,28]) leads to profound increases in blood catecholamines and arterial pressure.[29] Thus the lack of any change in these blood variables at least would diminish the possibility of the cerebral cortical "stimulation" being due to an increased sympathetic activity. This is supported further by the finding of Sakabe *et al.*,[5] that the N_2O induced increase in CBF and cerebral metabolism in dogs was not at all altered by pretreatment with reserpine. Reserpine (introduced systemically) has been shown to lead to a nearly complete depletion of cerebral catecholamines.[30]

Qualitatively, present results support the findings of an increase in cerebral metabolic rate and blood flow during N_2O exposure previously reported in dogs.[1–6]

TABLE 2. Cerebral Cortical Metabolic Rate for Oxygen ($CMRO_2$) Arteriovenous Differences in Oxygen Content ($C[a\text{-}v]O_2$) and Cortical Cerebral Blood Flow (CBF)

	$CMRO_2$ ($ml \cdot 100\ g^{-1} \cdot min^{-1}$)	C (a-v) O_2 ($ml \cdot dl^{-1}$)	Cortical CBF* ($ml \cdot 100\ g^{-1} \cdot min^{-1}$)
Awake Control	2.41 ± 0.13	4.8 ± 0.2	50.7 ± 1.7
60 min 70% N_2O/30% O_2	4.10† ± 0.33	4.0‡ ± 0.2	105.7† ± 9.9

* Calculated as an average of parietal and frontal cortex flows.
† $P < 0.001$.
‡ $P < 0.01$.
Number of observations = 14.
All values are x ± SEM.

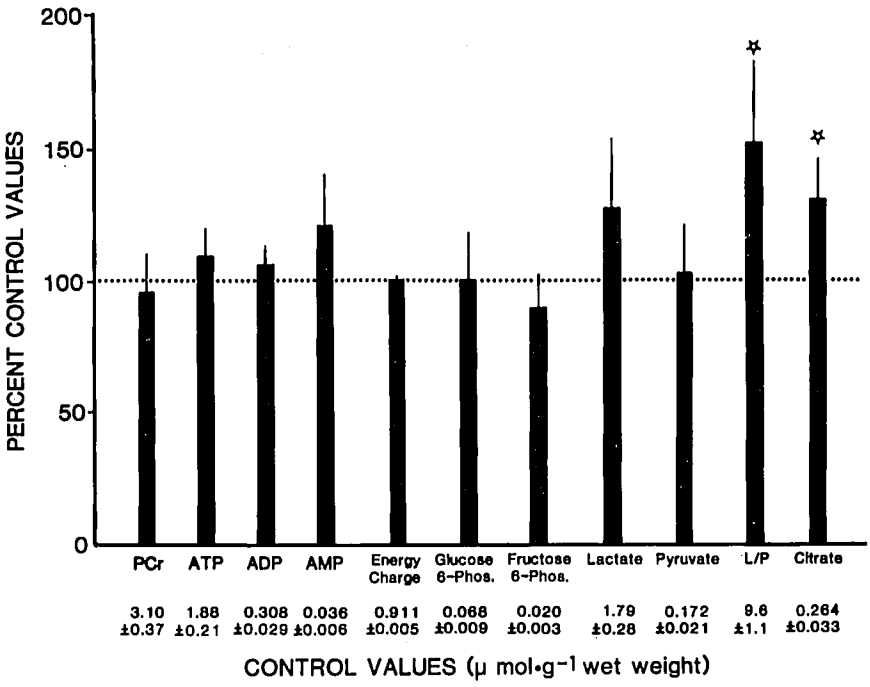

FIG. 3. Cerebral cortical metabolite levels (expressed as per cent control) at 60 min of N_2O exposure (19 paired observations). Control values (in $\mu mol \cdot g^{-1}$ wet weight ± SEM) are shown along the bottom. See figure 2 for symbol definition.

However, a quantitative comparison of results reveals that the increases in these variables in the present study were of a greater magnitude. That is, we found $CMRO_2$ to increase 70% in the presence of N_2O as compared with an 11–20% increase in dog studies. At first inspection, the total CBF increase recorded at 60 min in the present study (143% of control) is similar in magnitude to that found in the dog (125–156% of control). However, the CBF methods utilized in the dog studies (venous outflow and modified Kety–Schmidt) primarily measure cortical flow in that sagittal venous blood is sampled (see Siesjö[31]). Thus, when comparing our cortical CBF increases (188–246% of control) to that found in dogs, a rather large difference can be noted. In a final analysis, the true magnitude of the N_2O effect in the dog studies cannot be assessed accurately because of the lack of appropriate awake controls. That is, these animals were subjected variously to spinal anesthesia, low-level halothane and paralysis (see below), and a considerable amount of surgical stress in the preparation for CBF evaluation. The last has been associated with a time related fall in CBF.[32]

A much greater variance is present when comparing our results with findings in rat studies. In freely breathing rats, 70% N_2O has been reported to have only minimal effects on CBF and cerebral metabolic rate.[10,11] Gibson and Duffy,[7] on the other hand, reported significant increases in CBF and $CMRO_2$ during 70% N_2O. The rats in the Gibson and Duffy study appeared, however, to be highly stressed in that their control Pa_{CO_2} values were at least 10 mmHg lower than what generally is accepted as normal for the awake rat. In immobilized (paralyzed and artificially ventilated) rats, glucose utilization was shown to be increased during 70% N_2O in a number of cerebral structures[8] but CBF was relatively unaffected.[10,33]

Most studies in humans have indicated that N_2O produces either no change or a slight depression in whole brain CBF and metabolic rate.[12–14] However, Sakabe *et al.*[34] reported an increase in the "cerebral blood flow equivalent" (*i.e.*, the inverse of the arterial–jugular venous blood O_2 content difference) in patients during exposure to 60% N_2O. It must be emphasized that all of the above studies in humans were conducted with a background of paralysis and artificial ventilation *and* either thiopental induction,[12] morphine sulfate,[14] or 0.8% halothane.[34] Because paralysis and artificial ventilation[10,35] and anesthetic background[5] can modify the effect of N_2O on CBF and cerebral metabolism, these results must be interpreted with caution.

Tissue metabolites were measured in an attempt to gain some clue as to potential sites at the cellular level for the influence of N_2O on cerebral metabolism (and CBF). The present findings of a lack of any appreciable effect of N_2O on cerebral glycolytic metabolites and labile phosphates confirms previous reports in mice[15] and rats.[9] However, an absence of changes in the levels of PCr, ATP, ADP, and AMP does not rule out any effect of N_2O on cerebral oxidative function and energy demand. Rosenthal *et al.*[36] reported, in midcollicular level lesioned rats (cerveau isolé preparation), an increased oxidation of cytochrome a, a_3 during N_2O exposure, suggesting an increased energy demand. We recently found evidence (*in vitro*, using a goat cerebral cortical mitochondrial preparation—unpublished observations), which suggested that the increased O_2 utilization may be due at least partly to a direct effect of N_2O on mitochondrial respiration. This may imply that part of the increased O_2 consumption was due to factors not directly related to energy demand, *i.e.*, changes in the mitochondrion itself.

The lack of agreement among studies regarding the influence of N_2O (60–70%) on cerebral metabolism and blood flow may be due to any number of factors. The most likely possibilities, however, are 1) that the N_2O effects on cerebral blood flow and metabolism are species related with dogs and goats being influenced to a much greater extent than man or rodents; and/or 2) that the influence of N_2O on these variables is altered as a result of paralysis and artificial ventilation, background anesthetics and other pharmacologic agents, stress, and factors related to the preparation preceding the period of N_2O evaluation.

Regarding the first possibility, one cannot completely rule out that the varied results are related to a difference in the anesthetic requirement of N_2O (MAC) among species. Subanesthetic levels of N_2O (and other inhalation anesthetics) have been associated with cerebral excitation.[37] Thus, any stimulatory effect of 60–70% N_2O on CBF and cerebral metabolism might occur more readily in animals exhibiting a higher MAC for N_2O. The MAC for N_2O in dogs[38] appears to be greater than that found for rodents[39] or humans.[40] However, one should be cautioned to avoid any firm conclusions, in that these results were derived from a rather limited number of observations. No information regarding MAC for N_2O in the goat is presently available. If the percentage of MAC is an important factor in determining the degree of brain "stimulation" resulting from N_2O exposure, then one might expect to see a greater effect during the transient immediately following introduction of N_2O than after blood:brain equilibration. The finding by Sakabe *et al.*[5] and in the present study of a higher CBF over the first 15 min of N_2O exposure than at 1 hour is supportive. However, as noted above, the preparation used by Sakabe *et al.* is associated with a deterioration of CBF over time.

Sakabe *et al.* also reported that the $CMRO_2$ and EEG pattern remained fairly constant throughout the period of N_2O exposure (0.2% halothane background). Furthermore, no obvious signs of excitation were observed in our goats (see "Results"). This included virtually no variation in the continuously monitored arterial pressure (complete data not shown). It is, therefore, inappropriate at present to employ the concept of variations in anesthetic requirements for N_2O among species to entirely explain the lack of agreement in the literature.

Clearly, in regard to the second possibility (*i.e.*, methodologic factors) the most desirable procedure for evaluating the influence of N_2O *alone* on cerebral metabolism and blood flow would be one in which the subjects were freely ventilating and unmedicated, sufficient time was allowed for recovery from whatever surgical preparation was needed (*i.e.*, no acute postsurgical trauma or lingering anesthetic effects), and indications of stress were demonstrably absent. This is precisely the protocol we observed with the present preparation.

In conclusion, we have shown that in goats N_2O has a profound effect on cerebral metabolism and blood flow. Furthermore, no other factor other than N_2O *alone* could account for these results. These findings suggest that caution must be exercised when interpreting changes in these variables in studies where N_2O is employed.

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References

1. Chapman AG, Nordström C-H, Siesjö BK: Influence of phenobarbital on carbohydrate and amino acid metabolism in rat brain. ANESTHESIOLOGY 48:175–182, 1978
2. Abdul-Rahman A, Dahlgren N, Ingvar M, Siesjö BK: Local versus regional cerebral blood flow in the rat at high (hypoxia) and low (phenobarbital anesthesia) flow rates. Acta Physiol Scand 106:53–60, 1979
3. Michenfelder JD, Theye RA: In vivo toxic effects of halothane on canine cerebral metabolic pathways. Am J Physiol 229:1050–1055, 1975
4. Theye RA, Michenfelder JD: The effect of nitrous oxide on canine cerebral metabolism. ANESTHESIOLOGY 29:1119–1124, 1968
5. Sakabe T, Kuramoto T, Inoue S, Takeshita H: Cerebral effects of nitrous oxide in the dog. ANESTHESIOLOGY 48:195–200, 1978
6. Oshito S, Ishikawa T, Tokutsu Y, Takeshita H: Cerebral circulatory and metabolic stimulation with nitrous oxide in the dog. Acta Anaesthesiol Scand 23:177–181, 1979
7. Gibson GE, Duffy JE: Impaired synthesis of acetylcholine by mild hypoxia or nitrous oxide. J Neurochem 36:28–33, 1981
8. Ingvar M, Abdul-Rahman A, Siesjö BK: Local cerebral glucose consumption in the artificially ventilated rat: Influence of nitrous oxide analgesia and of phenobarbital anesthesia. Acta Physiol Scand 109:177–185, 1980
9. Nilsson L, Busto R: Effects of anesthesia on the energy and acid–base status of rat brain. Acta Anaesthesiol Scand 17:119–128, 1973
10. Dahlgren N, Ingvar M, Yokoyama H, Siesjö BK: Influence of nitrous oxide on local cerebral blood flow in awake, minimally restrained rats. J Cereb Blood Flow Metabol 1:211–218, 1981
11. Ingvar M, Siesjö BK: Effects of nitrous oxide on local cerebral glucose utilization in rats. J Cereb Blood Flow Metabol 2:481–486, 1982
12. Wollman H, Alexander SC, Cohen PH, Smith TC, Chase PE, von der Molen RA: Cerebral circulation during general anesthesia and hyperventilation in man. Thiopental induction to nitrous oxide and *d*-tubocurarine. ANESTHESIOLOGY 26:329–334, 1965
13. Smith AL, Hoffman SC, Wollman H: Effect of general anesthesia on auto-regulation of cerebral blood flow in man. J Appl Physiol 29:665–669, 1970
14. Jobes DR, Kennel EM, Bush GL, Mull TD, Lecky JH: Cerebral blood flow and metabolism during morphine nitrous oxide anesthesia in man. ANESTHESIOLOGY 47:16–18, 1977
15. Ratcheson RA, Bilezikjian L, Ferrendelli JA: Effects of nitrous oxide anesthesia upon cerebral energy metabolism. J Neurochem 28:223–225, 1977
16. Reimann C, Lluch S, Glick G: Development and evaluation of an experimental model for the study of cerebral circulation in the unanesthetized goat. Stroke 3:322–328, 1972
17. Miletich DJ, Ivankovic AD, Albrecht RF, Toyooka ET: Cerebral hemodynamics following internal maxillary ligation in the goat. J Appl Physiol 38:942–945, 1975
18. Pelligrino DA, Miletich DJ, Albrecht RF, Visintine D, Ripper R, Domninguez G, Kuse J: Evaluation of a system for serial biopsy of cerebral cortical tissue in an awake goat. Am J Physiol, 1984 (in press)
19. Passon PG, Peuler JD: A simplified technique for plasma norepinephrine and epinephrine. Anal Biochem 51:618–632, 1973
20. Heymann MA, Payne BD, Hoffman JIE, Rudolph AM: Blood flow measurement with radionuclide-labelled particles. Prog Cardiovasc Dis 20:55–79, 1977
21. Lowry OH, Passonneau JV: A Flexible System of Enzymatic Analysis. New York, Academic Press, 1972
22. Folbergrová J, MacMillan V, Siesjö BK: The effect of moderate and marked hypercapnia upon the energy state and upon the cytoplasmic NADH/NAD+ ratio of the rat brain. J Neurochem 19:2497–2505, 1972
23. Folbergrová J, MacMillan V, Siesjö BK: The effect of hypercapnic acidosis upon some glycolytic and Krebs cycle-associated intermediates in the rat brain. J Neurochem 19:2507–2517, 1972
24. Folbergrová J, Pontén U, Siesjö BK: Pattern of changes in brain carbohydrate metabolites, amino acids and organic phosphates at increased carbon dioxide tension. J Neurochem 22:1115–1125, 1974
25. Folbergrová J, Ljunggren B, Siesjö BK: Influence of complete ischemia on glycolytic metabolites, citric acid cycle intermediates, and associated amino acids in the rat cerebral cortex. Brain Res 80:265–279, 1974
26. Bahlman SH, Eger EI, Smith NT, Stevens WC, Shakespeare TF, Sawyer DC, Halsey MJ, Cromwell TH: The cardiovascular effects of nitrous oxide–halothane anesthesia in man. ANESTHESIOLOGY 35:274–285, 1971

27. Millar RA, Warden JC, Cooperman LH, Price HL: Central sympathetic discharge and mean arterial pressure during halothane anaesthesia. Br J Anaesth 41:918–928, 1969
28. Fukunaga AF, Epstein RM: Sympathetic excitation during nitrous oxide–halothane anesthesia in the cat. ANESTHESIOLOGY 39:23–26, 1973
29. Yamaguchi I, Kopin JJ: Plasma catecholamine and blood pressure responses to sympathetic stimulation in pithed rats. Am J Physiol 237:H305–310, 1979
30. Olson EB, Dempsey JA, McCrimmon DR: Serotonin and the control of ventilation in awake rats. J Clin Invest 64:689–693, 1979
31. Siesjö BK: Brain Energy Metabolism. New York, John Wiley and Sons, 1978, pp 56–100
32. Takeshita H, Michenfelder JD, Theye RA: The effects of morphine and N-allylnormorphine on canine cerebral metabolism and circulation. ANESTHESIOLOGY 37:605–612, 1972
33. Carlsson C, Hägerdal M, Siesjö BK: The effect of nitrous oxide on oxygen consumption and blood flow in the cerebral cortex of the rat. Acta Anaesthesiol Scand 20:91–95, 1976
34. Sakabe T, Kuramoto T, Kumagae S, Takeshita H: Cerebral responses to the addition of nitrous oxide to halothane in man. Br J Anaesth 48:957–962, 1976
35. Tommasino C, Shapiro HM: Effect of nitrous oxide analgesia on local cerebral glucose utilization during immobilization stress. J Cereb Blood Flow Metabol 3(Suppl 1):S572–S573, 1983
36. Rosenthal M, Adams MR, LaManna JC: Nitrous oxide alters oxidative metabolic activities of rat neocortex in situ. Brain Res 213:405–414, 1981
37. Winters WD, Forer-Alado T, Guzman-Florec C, Alcaraz M: The cataleptic state induced by ketamine: A review of the neuropharmacology of anesthesia. Neuropharmacology 11:303–315, 1972
38. Eger EI, Brandstater B, Saidman LJ, Regan MJ, Severinghaus JW, Munson ES: Equipotent alveolar concentrations of methoxyflurane, halothane, diethyl ether, fluroxene, cyclopropane, xenon and nitrous oxide in the dog. ANESTHESIOLOGY 26:771–777, 1965
39. Miller KW, Paton WDM, Smith EB, Smith RA: Physico-chemical approaches to the mode of action of general anesthetics. ANESTHESIOLOGY 36:339–351, 1972
40. Eger EI: Anesthetic uptake and action. Baltimore/London, Williams and Wilkins, 1974, pp 1–25