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Cerebrovascular and Cerebral Metabolic Effects of N_2O in Unrestrained Rats

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There is controversy about whether N_2O increases cerebral blood flow and cortical oxygen consumption ($CMRO_2$) in rats. Cortical and subcortical blood flow and $CMRO_2$ were measured in awake, unrestrained rats while awake and during 70% N_2O administration using radioactive microspheres. In the awake state, cortical and subcortical blood flow were 126 ± 10 and 98 ± 7 ml·100 g^{-1}·min^{-1}, respectively, and $CMRO_2$ (cortical) was 10.0 ± 0.6 ml O_2·100 g^{-1}·min^{-1} (mean ± SE). After 15 min of 70% N_2O, cortical and subcortical blood flow increased 100% and 40%, respectively, while $CMRO_2$ did not increase significantly. Cerebral blood flow remained increased after 60 min of N_2O exposure, and $CMRO_2$ did not change. These results show that N_2O produces cerebrovasodilation in rats that is not related to a change in metabolic demand. Plasma catecholamines do not change during N_2O administration, indicating that the increase in blood flow is not due to a general stress response. (Key words: Anesthetics: gases; nitrous oxide. Brain: blood flow; metabolism. Sympathetic nervous system, catecholamines: dopamine, epinephrine, norepinephrine.)

RAT CEREBRAL PHYSIOLOGY may be different from other species in its response to N_2O. In dogs, goats, rabbits, and humans, it has been shown that N_2O increases cerebral blood flow (CBF) and oxygen consumption ($CMRO_2$)[1–5]; however, in rats N_2O may not increase either CBF or brain metabolism.[6–9] One problem with these studies was that unanesthetized rats were restrained for blood flow and metabolism measurements. Bryan *et al*[10] have shown that stress associated with restraint increases brain glucose metabolism markedly compared to unrestrained rats. It is possible that stress associated with restraint also increased CBF in previous rat studies. It was the purpose of these experiments to evaluate the effect of N_2O inhalation on CBF and $CMRO_2$ in the unanesthetized, unrestrained rat.

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Materials and Methods

These experiments were carried out after approval from the Michael Reese Institutional Review Board for Animal Research. Male Sprague-Dawley rats weighing 450–500 g were anesthetized in a bell jar with isoflurane. Following tracheal intubation, their lungs were ventilated with 1.8% isoflurane in oxygen using a small animal ventilator. Catheters were inserted into both femoral arteries and into the left ventricle *via* the right carotid artery. These catheters were tunneled underneath the skin and exited from a small incision on the back. The skull was then exposed, a small hole drilled over the sagittal sinus, and a catheter inserted into the sinus and cemented in place using dental cement and a small screw in the skull. All incisions were infiltrated with bupivacaine and closed, a rectal thermistor was inserted and taped to the tail, and isoflurane was discontinued. The trachea was extubated when the animal was awake, and each rat was then allowed 2 h to recover from the effects of anesthesia.

In group 1 (n = 10), CBF and $CMRO_2$ were evaluated initially in unrestrained, awake rats. Each rat was placed in an airtight styrofoam chamber that was 30 cm long, 8 cm wide, and 10 cm high with a clear plexiglass top. The chamber was ventilated with 70% N_2/30% O_2 at a rate of 3 l/min using an Ohio anesthetic machine. The rectal probe and catheters were threaded through a small hole in the plexiglass top of the chamber. The rat was acclimatized in this chamber for 30 min. Chamber gases were sampled by a Datex anesthetic gas analyzer at a rate of 200 ml/min. Arterial blood pressure was monitored continuously throughout the experiment *via* the femoral artery catheter. At the end of the chamber equilibration period, the first CBF evaluation was performed using radioactive microspheres. Arterial and sagittal sinus blood samples were drawn for measurement of blood gas tensions, arterial and sagittal sinus O_2 content, and plasma catecholamines (total volume = 1.4 ml).

The inspired gases were then changed to 70% N_2O/30% O_2. The chamber environment was adjusted to 68–70% N_2O within 1 min by a rapid infusion of gases. After an additional 15-min equilibration period, a second CBF/$CMRO_2$ determination was made. A third determination was performed after 60 min of N_2O exposure. A second plasma catecholamine sample was drawn at this time.

To determine whether CBF and $CMRO_2$ changes are a function of the order of testing, a second group of rats was studied (n = 10). In these animals CBF and $CMRO_2$

were measured twice under control conditions (70% N_2/30% O_2) 15 and 60 min following a 30-min equilibration period.

MICROSPHERES

Fifteen-micrometer microspheres labeled with Co-57, Sn-113, and Sc-46 (New England Nuclear) were used. Stock solutions containing 500,000 microspheres/ml were suspended in isotonic saline with 0.01% Tween-80. Microspheres were vortexed for 1 min, and 0.2 ml was withdrawn (100,000 microspheres) and injected *via* the right carotid artery catheter into the left ventricle (dead space = 0.06 ml), which was flushed with 0.2 ml saline over 20 s. Starting immediately before each microsphere test and continuing 45 s after the end of each injection, blood was withdrawn from a femoral artery at 0.4 ml/min. Mean arterial blood pressure was measured continuously from the other femoral artery to ensure blood pressure did not change appreciably during the microsphere tests. Each rat was killed, and the brain was 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 calculated from gamma radiation activity according to the methods of Heymann *et al.*[11]

Arterial and sagittal sinus blood samples were taken following each CBF measurement for blood gas, *p*H, and oxygen content determinations. Blood gas tensions were measured with an Instrumentation Laboratories 1303 blood gas analyzer. Arterial-sagittal sinus O_2 content was calculated from the hemoglobin concentration, the oxygen saturation, and the amount of dissolved oxygen (calculated from Pa_{O_2} and oxygen solubility). Cortex oxygen consumption was calculated in each rat by multiplying cortex blood flow by the arterial minus sagittal sinus O_2 content. It has previously been shown in the rat that the sagittal sinus drains primarily cerebral cortex tissue, with little contribution from subcortex or extracerebral sources.[12]

Epinephrine, norepinephrine, and dopamine concentrations were measured from plasma samples taken in the awake period immediately before the start of testing and after the last CBF measurement in N_2O ventilated rats. Catecholamines were assayed according to previously reported methods.[13]

STATISTICS

Data are reported as mean ± SE. Changes in arterial blood gases, CBF, and $CMRO_2$ under 70% N_2 and 70% N_2O were evaluated using a one-way repeated measures ANOVA. Post-hoc comparisons between treatment conditions were made when appropriate using a Scheffe test with corrections for multiple comparisons.

Results

Mean arterial blood pressure, heart rate, and arterial blood gas tensions in unanesthetized (70% N_2) and treated rats (70% N_2O) are shown in table 1. There were no significant changes in blood pressure or blood gases after either 15- or 60-min N_2O exposure compared to values during 70% N_2 exposure (group 1). There was no difference in rectal temperature in any group. Plasma epinephrine, norepinephrine, and dopamine concentrations in rats exposed to 70% N_2 were 186 ± 20 pg/ml, 218 ± 14 pg/ml, and 45 ± 13 pg/ml, respectively. In N_2O exposed rats, epinephrine, norepinephrine, and dopamine concentrations were 238 ± 29 pg/ml, 346 ± 22 pg/ml, and 52 ± 11 pg/ml, respectively. There was no significant difference in catecholamine concentrations between N_2 and N_2O treatment conditions ($P > 0.10$).

Cortical and subcortical blood flow were similar during N_2 ventilation in groups 1 and 2. In group 1 cortex and subcortex flow increased 100% and 40%, respectively, 15

TABLE 1. Mean Arterial Blood Pressure, Blood Gas Tensions, Cerebral Blood Flow (CBF), and Cortical Oxygen Consumption ($CMRO_2$)

	N	Blood Pressure (mmHg)	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	*p*H	Cortex CBF	Subcortex CBF	$CMRO_2$
Group 1	10							
70% N_2		112 ± 3	38.8 ± 0.8	144 ± 4	7.41 ± .01	127 ± 10	98 ± 7	10.0 ± 0.6
70% N_2O (15)*		114 ± 3	37.7 ± 0.8	145 ± 3	7.42 ± .01	252 ± 41†	138 ± 17†	12.3 ± 1.6
70% N_2O (60)*		112 ± 3	38.1 ± 1.0	134 ± 5	7.42 ± .01	203 ± 28†	136 ± 13†	12.2 ± 1.5
Group 2	10							
70% N_2 (15)*		127 ± 5	38.5 ± 0.5	135 ± 5	7.41 ± .01	134 ± 14	95 ± 7	10.8 ± 1.6
70% N_2 (60)*		130 ± 7	37.0 ± 0.8	131 ± 4	7.42 ± .01	124 ± 11	93 ± 7	9.3 ± 0.6

Data reported as mean ± SE.

$CMRO_2$ was not measured in two rats in group 1 and one rat in group 2 due to loss of sagittal sinus blood sample.

CBF given as ml·100 g^{-1}·min^{-1}, $CMRO_2$ given as ml O_2·100 g^{-1}·min^{-1}.

* Numbers in parentheses represent duration (min) of N_2O or N_2 administrations.

† $P < 0.05$ compared with 70% N_2 within each group.

min after the initiation of N_2O ventilation and remained increased for 1 h during N_2O administration. The $CMRO_2$ was 10 ml $O_2 \cdot 100\ g^{-1} \cdot min^{-1}$ during N_2 ventilation and did not change significantly during N_2O administration. In group 2, which evaluated whether CBF or $CMRO_2$ changes were a function of the order of testing, there were no changes in blood flows or $CMRO_2$ over time during N_2 ventilation.

Discussion

In these experiments we showed that CBF increases in unrestrained rats spontaneously breathing 70% N_2O *versus* 70% N_2. The 100% increase in CBF produced by N_2O is consistent with similar results observed in goats[2] and in humans.[14,¶] Gibson and Duffy[15] have also reported that N_2O increases CBF 70% in rats compared to unanesthetized controls. In our study $CMRO_2$ increased 20% ($P > 0.05$) during N_2O. This greater increase in CBF than in $CMRO_2$ agrees with earlier reports in dogs and goats where N_2O increased CBF and decreased oxygen extraction.[1-3] Our data are also consistent with the results of Crosby *et al.*[16] who showed that muscle paralysis and N_2O ventilation in rats will produce a 15–25% increase in brain glucose metabolism compared to conscious animals. The inability to measure CBF changes with N_2O in earlier rat studies[6,8] may be due to the use of restraint in unanesthetized animals, which can produce a stress response.[13]

It is known that stress can produce marked cerebrovascular and cerebral metabolic changes. The rats in our experiments were tested 2 h after recovery from surgical anesthesia. Although they appeared unstressed in the testing environment, plasma catecholamine concentrations were not as low as have been reported in rats after several days recovery from surgery and anesthesia.[10,13] This suggests that our baseline CBF and $CMRO_2$ values may not represent a completely unstressed state. Bryan *et al.*[10] showed that following several days of recovery, epinephrine concentrations were in the range of 50 pg/ml in unanesthetized, unrestrained rats. Restraint increased this concentration to over 1000 pg/ml and increased brain glucose metabolism by 50–100%. It is possible that a longer recovery period in our study would decrease resting control values of both CBF and $CMRO_2$. However, it is unlikely that the increase we report in CBF produced by N_2O was due to a stress response since plasma catecholamine concentrations did not change during N_2O administration.

While it is difficult to compare data from several studies, the difference in CBF between unanesthetized rats and rats breathing N_2O in this experiment agrees with other data from our laboratory. We previously reported an unanesthetized rat brain blood flow of approximately 100 $ml \cdot 100\ g^{-1} \cdot min^{-1}$.[17] In rats treated with muscle relaxants whose lungs were mechanically ventilated with N_2O, cortical blood flows of 150 to 200 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ have been measured.[18-20] Our data confirm previous reports that N_2O produces a prolonged increase in CBF that lasts at least 1 h.[1-4,7] However, the attenuation of this vasodilatory effect over time[2] may account for differences in control CBF values previously reported by others during N_2O administration.[8,9,11] We are unaware of previous unanesthetized and unrestrained $CMRO_2$ measurements in rats. However, $CMRO_2$ values of 10–11 ml $O_2 \cdot 100\ g^{-1} \cdot min^{-1}$ have been reported consistently in paralyzed rats breathing N_2O.[6,18-20] It is difficult to evaluate the effect of muscle relaxation, postsurgical stress, and length of equilibration in these studies.

In summary, we showed that in unanesthetized and unrestrained rats, N_2O will produce a significant increase in CBF and a nonsignificant increase in $CMRO_2$. The cerebrovascular effects of N_2O were not associated with significant changes in blood gas tensions, arterial blood pressure, or plasma catecholamine concentrations. The increase in CBF produced by N_2O in rats is consistent with reports in other species, including dogs, goats, rabbits, and humans.[1-5,14] Although cerebral autoregulation and cerebrovascular responses to CO_2 are maintained during N_2O,[21] it is possible that N_2O may have a direct effect on cerebral vessels to increase CBF. Nitrous oxide may also change neurotransmitter activity in the brain, decreasing neurovascular tone and increasing CBF to regions such as the cortex. The mechanism by which N_2O increases CBF is not known at present.

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