Inhibition of cerebral metabolic and circulatory responses to nitrous oxide by 6-hydroxydopamine in dogs

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Purpose: To determine whether cerebral metabolic and circulatory consequences of N₂O result from activation of the sympathoadrenal system. The effects of pretreatment with intracisternal injection of 6-OHDA, which produces chemical sympathectomy, were studied in dogs.

Method: Seven days before measurement dogs were pretreated with intracisternal injection of either saline vehicle (sham-group) or 100 µg·kg⁻¹ 6-hydroxydopamine (6-OHDA group). Cerebral blood flow (CBF) was measured using an electromagnetic flow-meter probe and cerebral metabolic rate for oxygen (CMRO₂) was calculated as the product of CBF and arterial-sagittal sinus blood oxygen content difference [C(a-v)O₂].

Results: In the sham group, N₂O (60%) increased CMRO₂ from 6.11 ± 0.21 ml·100 g⁻¹·min⁻¹ to 7.10 ± 0.39 ml·100 g⁻¹·min⁻¹ and CBF from 63 ± 5 ml·100 g⁻¹·min⁻¹ to 173 ± 26 ml·100 g⁻¹·min⁻¹. In the 6-OHDA group, CMRO₂ did not change during N₂O exposure, whereas CBF increased from 61 ± 3 ml·100 g⁻¹·min⁻¹ to 135 ± 19 ml·100 g⁻¹·min⁻¹ but less than in the sham group. The 6-OHDA group displayed a reduction in cortical noradrenaline (NA) concentration from 263.2 ± 35.6 ng·g⁻¹ to 102.7 ± 16.5 ng·g⁻¹. Cortical dopamine (DA) concentration was not affected by 6-OHDA administration.

Conclusion: These results suggest that most of the increase in CMRO₂ and, at least a part of, the increase in CBF during N₂O exposure in the sham-group are related to sympathoadrenal-stimulating effects of N₂O.

Objectif : Vérifier si les effets métaboliques et circulatoires cérébraux provoqués par l'inhalation de N₂O résultent de l'activation du système sympathico-surrénalien. Au cours de cette étude, on a étudié sur des chiens les effets de la sympathectomie chimique provoquée par l'administration intracisternale de 6-OHDA.

Méthodes : D'abord, les chiens ont reçu des injections intracisternales de sol. phys. (Groupe factice) ou de 100 µg·kg⁻¹ de 6-hydroxydopamine (groupe 6-OHDA). Sept jours plus tard, le débit sanguin cérébral (DSC) a été mesuré à l'aide d'un débitmètre électromagnétique ; le métabolisme cérébral pour l'oxygène (CMRO₂) a été calculé en multipliant le DSC par la différence artério-sinus sagittal du contenu en oxygène [C(a-v)O₂].

Résultats : Dans le groupe factice, le N₂O (60%) a augmenté le CMRO₂ de 6,11 ± 0,21 ml·100 g⁻¹·min⁻¹ à 7,10 ± 0,39 ml·100 g⁻¹·min⁻¹ et le DSC de 63 ± 5 ml·100 g⁻¹·min⁻¹ à 173 ± 26 ml·100 g⁻¹·min⁻¹. Dans le groupe 6-OHDA, le CMRO₂ n'a pas varié pendant l'exposition au N₂O, alors que le DSC augmentait de 61 ± 3 ml·100 g⁻¹·min⁻¹ à 135 ± 19 ml·100 g⁻¹·min⁻¹ mais moins que dans le groupe factice. Dans le groupe 6-OHDA, la concentration de la noradrénaline corticale baissait de 263,2 ± 35,6 ng·g⁻¹ à 102,7 ± 16,5 ng·g⁻¹. L'administration de 6-OHDA n'a pas affecté la concentration de la dopamine corticale.

Conclusion : Ces résultats suggèrent que, dans le groupe factice, pendant l'exposition au N₂O, la presque totalité de l'augmentation du CMRO₂ et, au moins une partie de l'augmentation du DSC, sont causées par la stimulation sympathico-surrénalienne.

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NITROUS oxide (N_2O) is an inhalational anaesthetic that both in humans¹⁻² and in experimental animals³⁻⁵ uniquely causes an increase in the cerebral metabolic rate for oxygen ($CMRO_2$) accompanied by an increase in cerebral blood flow (CBF) and/or intracranial pressure (ICP). Although the precise mechanisms by which N_2O increases $CMRO_2$ and CBF are not clear, Millar *et al.*,⁶ Bahlman *et al.*,⁷ and Fukunaga and Epstein⁸ reported that, in both cats and human volunteers, N_2O had a stimulating effect on the sympathoadrenal system, an action that may originate at suprapontine levels.⁸ In addition, Edwards *et al.*⁹ reported that intravenous injection of catecholamines into four to six day old chicks enhanced cerebral phosphorylase activity with a resulting breakdown of glycogen. Sokrab and Johansson¹⁰ showed that intravenous infusion of epinephrine increased CBF in conscious rats as a result of enhanced cerebral metabolism. Six-hydroxydopamine (6-OHDA) is a neurotoxin specific for catecholaminergic neurons,¹¹ and Edvinsson *et al.*¹² reported that intraventricular injection of 6-OHDA led to marked degeneration of catecholaminergic nerve terminals throughout the brain.

These findings suggest that the cerebral metabolic and circulatory effects of N_2O might result from activation of the sympathoadrenal system. To examine this hypothesis, we previously studied the effects, in dogs, of pretreatment with reserpine on cerebral metabolic and circulatory responses to N_2O .³ In that study,³ the dogs were pretreated with $0.5 \text{ mg}\cdot\text{kg}^{-1}$ reserpine *im* for two days before administration of N_2O , but the reserpine did not modify the effects of N_2O on $CMRO_2$ and CBF. We concluded that sympathoadrenal stimulation during N_2O exposure was not the mechanism leading to increases in the studied variables.³ However, we could not exclude the possibility that the dogs were incompletely depleted of catecholamines. To determine the role of the sympathoadrenal system in cerebral metabolic and circulatory responses to N_2O , the present study was designed to evaluate the effects of pretreatment of dogs anaesthetized with halothane with intracisternal injection 6-OHDA on N_2O -induced increases in both $CMRO_2$ and CBF. We confirmed that, as shown by Stahl *et al.*¹³ intracisternal administration of 6-OHDA caused chemical sympathectomy via degeneration of catecholamine-containing neurons in the central nervous system without changes in circulating catecholamines.

Materials and methods

The protocol was approved by the animal investigation committee of the institution. Fourteen unmedicated mongrel dogs, male and female, weighing 8–16 kg, were anaesthetized with intraperitoneal $50 \text{ mg}\cdot\text{kg}^{-1}$

pentobarbital, then mounted in a stereotaxic apparatus. Seven dogs received intracisternal injection of $100 \text{ }\mu\text{g}\cdot\text{kg}^{-1}$ 6-OHDA, 6-OHDA group). The other seven dogs were treated identically except that the same amount of saline vehicle (1 ml) without 6-OHDA was injected intracisternally (sham-operated group). Then, all dogs were returned to their cages and allowed to recover for seven days.

On the day of experiment, dogs in both groups were anaesthetized with halothane (2–3%) in oxygen. The trachea was intubated and the lungs were ventilated through a volume-limited Harvard pump (model 683). Muscle relaxation was maintained with $110\text{--}130 \text{ mg}\cdot\text{hr}^{-1}$ succinylcholine. During surgery, halothane (1–1.5%) was maintained in a gas mixture of nitrogen (60%) and oxygen (40%). Cannulas were placed in a femoral artery for blood sampling and pressure measurements, and in a femoral vein for drug administration and fluid infusion. Direct measurement of cerebral blood flow (CBF) was carried out using an electromagnetic flow-meter probe (lumen diameter 3 mm) incorporated 1 cm from the draining portion of the sinus.¹⁴ Briefly, after the dogs were heparinized by an initial dose of $3 \text{ mg}\cdot\text{kg}^{-1}$ ($1 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{hr}^{-1}$, subsequently), the sagittal sinus was cannulated through bone and the drained blood, which is the venous drainage from the anterior, superior, and lateral portions of both cerebral hemispheres, was returned to the left external jugular vein through the draining cannula. After completion of the surgical procedure, the dogs were placed in the prone position on a heating pad. Lidocaine ($5 \text{ mg}\cdot\text{kg}^{-1}$ 0.5% solution) was injected into the skin and muscles of the head and of the area where the cannula was placed. Additional lidocaine (half the initial dose) was given hourly. Halothane in the inhaled gas mixture was reduced to 0.2% end-tidal concentration and was maintained for the remainder of the study. The end-tidal concentrations of halothane and N_2O were measured by gas chromatography (Shimadzu GC-4A-TF).

The measurements commenced 50–90 min after completion of the surgery. Cerebral blood flow was determined using an electromagnetic flow-meter probe (lumen diameter 3 mm) incorporated 1 cm from the draining portion of the sinus.¹⁵ The volume of blood passing through the brain and the individual brain weights of the dogs were used to convert units of flow to $\text{ml}\cdot 100 \text{ g}^{-1} \text{ brain}\cdot\text{min}^{-1}$, as described by Michenfelder *et al.*¹⁴ Arterial PO_2 , PCO_2 , and pH, and sagittal sinus PO_2 values were measured with a radiometer ABL 300 blood gas analyzer at 37°C . Oxygen saturation and haemoglobin concentration were measured spectrophotometrically with an IL Co-oximeter (model 482). The oxygen content was calculated from the oxygen satura-

tion, haemoglobin concentration, and the amount of dissolved oxygen (estimated from PO_2 , and oxygen solubility). Cerebral metabolic rate for oxygen ($CMRO_2$) was calculated as the product of CBF and arterial-sagittal sinus blood oxygen content difference [$C(a-v)O_2$]. In both groups, control measurements were obtained over 30 min before the administration of N_2O , and the average values of CBF and $CMRO_2$ were calculated from five to eight consecutive determinations. The CBF and $CMRO_2$ were determined repeatedly over 120 min; this period was divided into two intervals of 60 min, the addition period and the withdrawal period (Figure 1). At the beginning of the addition period, N_2O was suddenly substituted for nitrogen. Sixty minutes later, nitrogen was substituted for N_2O . The CBF and $CMRO_2$ were measured at 5, 10, 15, 30, 45 and 60 min from the start of each period.

Immediately after CBF and $CMRO_2$ measurements, arterial and central venous pressures were measured. Ventilation and FiO_2 were adjusted to maintain $PaO_2 > 170$ mmHg and $PaCO_2$ at 36 ± 2 mmHg (mean SEM). Sodium bicarbonate was given as needed to keep the buffer base normal. Epidural temperature was measured with a calibrated thermistor probe and was maintained at $37.0 \pm 0.5^\circ C$. The haemoglobin concentration was maintained at 12.1 ± 0.3 g·dl⁻¹.

In five sham-operated and four 6-OHDA-treated dogs, tissue samples of cerebral cortex were obtained at the conclusion of the experiments, and noradrenaline and dopamine concentrations in the cerebral cortex were determined as described below. Tissue samples were frozen immediately after isolation, then stored at $-40^\circ C$ until analyzed using high-performance liquid chromatography (Shimadzu LC-1) with electrochemical detection (Bioanalytical System LC3A). Samples were weighed, homogenized in 0.1M perchloric acid, absorbed over activated alumina and Tris buffer, washed three times with 1.0 ml of nanopure water, then eluted with 200 ml 0.1 M perchloric acid. Fifty μ l of supernatant were then introduced at the top of the chromatography column. The lower limit of detection of the assay for the catecholamines was approximately 20 pg·ml⁻¹, with an overall coefficient of variation of 5~10%.

Statistical analyses were performed by two-way analysis of variance with critical difference-testing; $P < 0.05$ was considered to be significant.

Results

Following introduction of N_2O (60%), the average mean arterial blood pressure (MAP) increased from 107 ± 7 mmHg (Mean $\pm$ SEM) to 127 ± 7 mmHg in the sham-operated group. In contrast, in the 6-OHDA group the averaged MAP did not change. In both groups, other physiological variables including arterial PO_2 , PCO_2 , and pH, and brain temperature did not change throughout the experiments (Table I).

There was no difference between groups in the control $CMRO_2$ and CBF values. In the sham-operated group, 60% N_2O , in the presence of 0.2% halothane, increased both $CMRO_2$ and CBF throughout the 60-min period; $CMRO_2$ increased from 6.11 ml·100g⁻¹·min⁻¹ to 7.10 – 7.34 ml·100g⁻¹·min⁻¹ during administration of N_2O , while CBF increased and reached its maximum 10 min after the administration of N_2O (from 63 ml·100g⁻¹·min⁻¹ to 173 ml·100g⁻¹·min⁻¹), and thereafter progressively declined, but was still 111 ml·100g⁻¹·min⁻¹ at 60 min. In the 6-OHDA group, $CMRO_2$ did not change during N_2O exposure, whereas CBF increased to a maximum 135 ml·100g⁻¹·min⁻¹ at 10 min ($P < 0.05$), then gradually declined to 84 ml·100g⁻¹·min⁻¹ from 61 ml·100g⁻¹·min⁻¹ at 60 min. The increases in CBF in the 6-OHDA group were less than those in the sham-operated group. In both groups, these cerebral metabolic and circulatory changes returned to control values within 10 min when nitrogen was substituted for N_2O (Table II).

Cortical catecholamine concentrations for the sham-operated and 6-OHDA-treated dogs used for $CMRO_2$ and CBF measurements are shown in the Figure. In 6-OHDA-treated dogs there was 60% depletion in cortical noradrenaline concentration compared with those in sham-operated dogs. Cortical dopamine concentrations were not affected by 6-OHDA administration.

TABLE I Physiological variables during N_2O addition and withdrawal period in dogs

Periods	Groups	MAP (mmHg)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH
Control	sham-op	107 ± 7	189 ± 8	34 ± 1	7.41 ± 0.01
	6-OHDA	102 ± 8	177 ± 11	36 ± 1	7.37 ± 0.03
N_2O addition	sham-op	127 ± 7	188 ± 7	35 ± 1	7.38 ± 0.01
	6-OHDA	110 ± 7	178 ± 7	36 ± 2	7.39 ± 0.02
N_2O withdrawal	sham-op	106 ± 6	190 ± 11	35 ± 1	7.41 ± 0.01
	6-OHDA	101 ± 5	178 ± 9	36 ± 3	7.39 ± 0.02

Mean $\pm$ SD of seven dogs.

TABLE II Modification of cerebral circulatory and metabolic Response to N₂O by pretreatment with 6-Hydroxydopamine in dogs

<i>Periods</i>	<i>Time</i>	<i>Groups</i>	<i>CBF</i> <i>(ml·100 g⁻¹·min⁻¹)</i>	<i>CMRO₂</i> <i>(ml·100 g⁻¹·min⁻¹)</i>
Control		sham-op*	63 ± 5	6.11 ± 0.21
		6-OHDA	61 ± 3	5.98 ± 0.07
		sham-op	173 ± 26 [†]	7.10 ± 0.39 [†]
N ₂ O addition	10 min	6-OHDA	135 ± 19 [†]	6.00 ± 0.20
	30 min	sham-op	141 ± 17 [†]	6.92 ± 0.28 [†]
		6-OHDA	92 ± 6 [†]	5.99 ± 0.23
		sham-op	111 ± 8 [†]	7.34 ± 0.43 [†]
	60 min	6-OHDA	84 ± 9	6.32 ± 0.07
	10 min	sham-op	65 ± 4	6.44 ± 0.19
6-OHDA		65 ± 7	5.95 ± 0.10	
N ₂ O withdrawal	30 min	sham-op	60 ± 4	6.16 ± 0.22
		6-OHDA	59 ± 4	5.94 ± 0.16
		sham-op	57 ± 3	6.11 ± 0.28
	60 min	6-OHDA	57 ± 14	5.81 ± 0.14

* Sham-op: Sham operated

Mean ± SD of seven dogs.

† different from control ($P < 0.05$). FIGURE Noradrenaline (NA) and dopamine (DA) concentrations in the cerebral cortex measured seven days after sham-operation (open bar, n=5) or intracisternal injections of 100 µg·kg⁻¹ of 6-OHDA (closed bar, n=4).* $P < 0.05$ vs catecholamine concentrations obtained in the sham-operated group.

Discussion

Nitrous oxide is a unique drug in that it may stimulate the central sympathetic neuronal system,¹⁶ and it may have an activating effect on the descending inhibitory system, which includes the central nora-

drenergic system originating from the locus coeruleus. 6-OHDA produced 80% depletion of cortical noradrenaline without affecting cortical dopamine by intraventricular injection.¹⁷ In addition, intraventricular injection of 6-OHDA led to a heterogeneous denervation pattern with very marked noradrenaline denervation in the nucleus locus ceruleus-innervated areas and to a gradual disappearance of noradrenaline with the maximum reduction occurring seven to ten days after 6-OHDA administration.¹⁸

During the administration of 60% N₂O in the presence of halothane 0.2%, CMRO₂ increased in the sham-operated group but did not change in the 6-OHDA group. In addition, in the 6-OHDA group, the percentage increase in CBF during N₂O exposure was smaller than that in the sham-operated group. These findings indicate that pretreatment with 6-OHDA attenuated a major part of the N₂O-induced increases in CMRO₂ and at least a part of N₂O-induced increases in CBF. Intracisternal injection of 6-OHDA produced 60% depletion of cortical noradrenaline without affecting cortical dopamine. The small discrepancy between these cortical noradrenaline depletion data may be due in part to differences in methodology and species, and in part to variability in body temperature, induction agent, or background anesthesia. These results strongly suggest that central noradrenergic neurons participate in the cerebral metabolic and circulatory stimulating effects of N₂O. Our previous failure to demonstrate modification by pretreatment with reserpine might have been due to incomplete depletion of central catecholamines.

Qualitatively, the present results obtained in sham-operated dogs confirm the increase in CMRO_2 and CBF during N_2O exposure previously reported from our laboratory.³ However, quantitative comparison of results revealed that the increases in CBF were of greater magnitude in the present study. That is to say, we found CBF to increase approximately 80–170% above control in the sham-operated group compared with the 35–110% increase in our previous studies.³ We have no satisfactory explanation for the differences between the results obtained in the present study and those of our previous studies. However, muscle paralysis and artificial ventilation, background anaesthesia, species, and CBF measurement method, all of which are thought to modify the effects of N_2O on CMRO_2 and CBF,⁴ were the same as in our previous studies.³ Thus, it seems most likely that the sham-operation intracisternal injection of saline *per se* might have enhanced the stimulating effects on CMRO_2 and CBF through the excitement of the sympathetic nerve by N_2O , although intracisternal injection of 6-OHDA sedates the sympathetic nerve.

This hypothesis is supported by the increased arterial blood pressure during N_2O exposure in the sham-operated dogs, whereas in our previous studies there was little change.³ Intracisternal injection may alter the autonomic response to N_2O inhalation, because these injections affect the nucleus tractus solitarius and the hypothalamus. We measured MAP before the intracisternal injection. There was very little difference between pretreated MAP and control MAP, which was measured before N_2O inhalation. Signs of autonomic abnormality and other lesions were not observed after the intracisternal injection. Autoregulation and the CO_2 response were kept at 50 mmHg to 150 mmHg cerebral perfusion pressure after the end of the experiment by using CO_2 3% inhalation and phenylephrine hydrochloride and by reduction of blood volume by venipuncture.

It is generally believed that the cerebral vascular effects of anaesthetics are primarily passive in response to metabolic effects rather than direct and active, i.e., that function drives metabolism and metabolism drives flow.¹⁷ However, in the 6-OHDA group, CBF increased to a maximum approximately 110% above control at 10 min after N_2O without increase in CMRO_2 (Table II). These results indicate that the increases in CBF with N_2O cannot be explained solely as a secondary response to cerebral metabolic changes, suggesting that N_2O is a direct and potent cerebral vasodilator in dogs.

In summary, the present study has provided new information on the influence of the noradrenergic system on cerebral metabolic events during N_2O exposure. It is tempting to conclude that the increase in CMRO_2

during N_2O exposure is related, at least in part, to enhanced release of noradrenaline at central synapses.

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