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Regional cerebral metabolic rate (positron emission tomography) during inhalation of nitrous oxide 50% in humans

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Background. Recent studies in man have shown that cerebral blood flow increases during inhalation of nitrous oxide (N_2O), a finding which is believed to be a result of an increased cerebral metabolic rate (CMR). However, this has not previously been evaluated in man.

Methods. Regional CMR_{glu} ($rCMR_{glu}$) was measured three dimensionally with positron emission tomography (PET) after injection of 2-(^{18}F)fluoro-2-deoxy-D-glucose in 10 spontaneously breathing men (mean age 31 yr) inhaling either N_2O 50% in O_2 30% or O_2 30% in N_2.

Results. Global CMR_{glu} in young men was 27 (3) μmol 100 g^{-1} min^{-1} [mean (SD)]. Inhalation of N_2O 50% did not change global CMR_{glu} [30 (5) μmol 100 g^{-1} min^{-1}] significantly, but it changed the distribution of the metabolism in the brain ($P<0.0001$ analysis of variance). Compared with inhalation of O_2 30% in N_2, N_2O 50% inhalation increased the metabolism in the basal ganglia [14 (17)%, $P<0.05$] and thalamus [22 (23) %, $P<0.05$]. There was a prolonged metabolic effect of N_2O inhalation seen on a succeeding PET scan with oxygen-enriched air ($P<0.0001$) performed 1 h after the N_2O administration.

Conclusions. Inhalation of N_2O 50% did not change global CMR_{glu}, but the metabolism increased in central brain structures, an effect that was still present 1 h after discontinuation of N_2O.

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Nitrous oxide (N_2O) has been used for anaesthesia during neurosurgical procedures for half a century, as it was previously thought to be inert.[1] However, in the 1970s, it was shown that N_2O had potentially striking effects on intracranial pressure (ICP).[2 3] The literature regarding the effects of N_2O on the brain is still equivocal, probably due to species differences, but also because of interactions with other drugs or interventions. In humans, evidence supporting the conclusion that N_2O is a cerebral vasodilator in the absence of other interventions has been obtained from both two- and three-dimensional cerebral blood flow (CBF) studies.[4–6] Three-dimensional CBF measurements during N_2O 50% inhalation show that CBF increased in all regions, even though not evenly distributed in the brain.[6] The reason for this increase in flow is still unknown, but it may theoretically be due to an increased cerebral metabolism[7] or an influence of N_2O on the cerebral vessels,[8] for instance through release of vasoactive mediators.

In order to explore the former hypothesis, the aim of the present study was designed to evaluate the effect of N_2O 50% on the cerebral metabolic rate for glucose (CMR_{glu}) and its distribution using a three-dimensional positron emission tomography (PET) technique.

Methods

Ten male volunteers, age 25–40 yr (mean 31 yr), participated in the study. The ethics committee for human

studies and the isotope committee at the University of Lund approved the protocol. Written informed consent was obtained from each participant.

The participants breathed spontaneously into a facemask held in place by a rubber head strap. After eliminating air leaks, the volunteers inhaled one of two different gas mixtures starting 15 min before and continued 35 min after the injection of 2-(^{18}F)fluoro-2-deoxy-D-glucose (2-^{18}FDG). The inhaled gas mixtures were oxygen-enriched air (O_2 30%) and a mixture of N_2 20%, O_2 30%, and N_2O 50%. Oxygen-enriched air was compressed air and O_2 mixed with flow meters (Unit 760, Siemens, Elema, Solna, Sweden) whereas N_2O 50% with O_2 30% in N_2 was delivered as a pre-mixed precision gas produced by Alfax (Malmö, Sweden). Each subject served as it own control, starting by either inhaling N_2O 50% or O_2 30% in a randomized order succeeded by the other gas mixture. A PET scan was performed in each situation within 60 min after the FDG injections. The results were evaluated after six investigations, and as the three subjects who inhaled N_2O 50% first had remaining effects of N_2O on the succeeding PET scans, all the rest of the investigations (four) were done by starting with O_2 30%. The data from the three subjects are included in the physiological values, but the results from their PET scanning are presented separately, and none of these data was included in the main study. Two volunteers breathed air twice in order to control the validity of the method. One participant had discomfort by inhaling N_2O and no data from this subject are included in any part of the study.

PET examinations were performed with a PC384-7B scanner (Scanditronix, Uppsala, Sweden). The four ring detector system generated four main slices and three cross slices, with an approximate slice thickness of 13 mm. The mean sensitivity of the scanner was 675 cps (kBq ml^{-1})$^{-1}$ for the main slices and 945 cps (kBq ml^{-1})$^{-1}$ for the cross slices. Corrections for random and scattered events were made. The attenuation was calculated from contours using a soft-tissue attenuation coefficient.

The 2-^{18}FDG was synthesized using^{18}F from the Lund electrostatic tandem accelerator with a proton beam of 6.0 MeV and produced by the ^{18}O(p,n)^{18}F reaction,[9] and by fluorination of the precursor 1,3,4,6-tetra-*O*-acetyl-2-*O*-trifluoro-methane-*b*-D-mannopyranose according to a method described by Toorongian and colleagues.[10] The radiochemical purity of FDG was better than 95%.

PET images were acquired within 60 min after administration of 50–150 MBq FDG, with the highest dose at the second measurement, by a 2 min i.v. injection into a peripheral vein in the arm. Venous blood samples were drawn from the other arm at appropriate intervals for quantification (every 20 s for 4 min, every 60 s for 10 min, and every 5 min up to a total of 35 min). When the blood samples had been centrifuged, radioactivity in the plasma (C_p) was measured using a well counter. Blood glucose (C_g) values were obtained, from the same site, immediately before, 10 and 35 min after injection of FDG. Two FDG studies were made on each subject with a time interval of approximately 2 h. The three-dimensional metabolic rate of glucose in the brain was recorded, parallel to the orbito-meatal (OM) line, with the centre of the lowest slice located 1 cm below the OM line. The head position was controlled using light beams on the external auditory meatus and the nasion as landmarks. Each study consisted of two separate scans (2×10 min), in between which the couch was moved half a plane separation (6.75 mm) in order to get a higher axial sampling rate. The CMR_{glu} were calculated using the test–retest method developed by Brooks and colleagues,[11] which takes into account the residual activity from the first investigation in the calculation of the second.

A number of blood samples were taken during a 35 min period after each FDG-injection, and the activity in plasma was measured. These curves were extrapolated by fitting a monoexponential function to the tail of the curve (the last 25 min). The first extrapolated curve was adjusted to match a sample taken immediately before the second injection. An example of a plasma activity curve is shown in Figure 1.

Tomographic images were reconstructed by filtered back projection using a Hanning reconstruction filter with a cut-off frequency of 2.1 cm^{-1}. The image matrix size was 128×128 and the pixel size 2.2 mm.

The calculation of the second measurement demanded that the images from the two studies were perfectly registered. This was accomplished first by carefully positioning the subjects in the same way for the two studies using line-laser for positioning, and secondly by shifting and rotating the images interactively.

Standard sets of regions of interest (ROIs) corresponding to the brain lobes, cerebellum, pons, thalamus, and basal ganglia were analysed by a ROI analysis program (Amersham, UK). The program works with anatomical templates from a CT brain atlas.[12] The templates were applied to the brain slices and scaled to the actual head

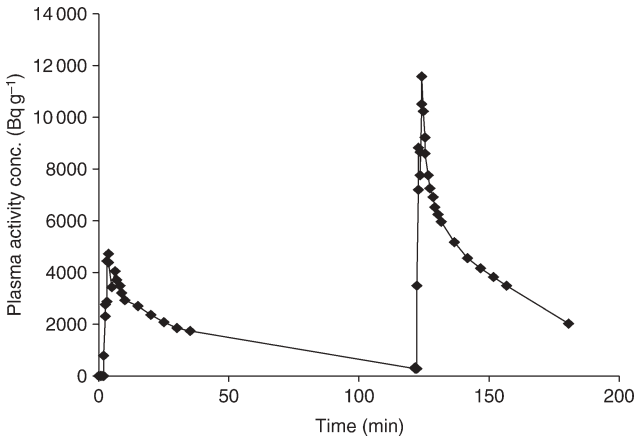

Fig 1 Representative curve from one participant demonstrating the activity of FDG in plasma as a function of time.

size according to the external brain diameters. Three-dimensional cerebral ROIs were calculated from adjoining ROIs in different brain slices representing the same structure. With the aid of the ROIs, the mean CMR_{glu} in different brain regions and the global mean CMR_{glu} was calculated.

The quantities of CO_2, O_2, and N_2O in the inspiratory and expiratory gas mixtures were continuously measured along with the pulse rate and arterial haemoglobin oxygen saturation (Sa_{O_2}), with an Ohmeda 4700 OxiCap (BOC Health Care, Louisville, KY, USA). Non-invasive arterial pressure was recorded with 5 min intervals (Colin Press-Mate, Colin Electronics, Japan) during the inhalation of gas. At the end of the study, a blood sample was withdrawn to measure the haemoglobin concentration.

Global effects of N_2O on the CMR_{glu} were analysed by two factor repeated measures analysis of variance (ANOVA) with correction for departure from sphericity for the interaction data.[13] As the investigations were test–retest situations with residual activity from the first investigation, this activity had to be subtracted analysing the second measurement. The increased noise level of the subtraction method, in combination with the limited number of subjects, will limit our findings to major CMR_{glu} changes in the order of 10–15% or more, whereas smaller changes will not be detected.

The effects of N_2O on the $rCMR_{glu}$ (ROI) was tested with primary component analysis (PCA) in order to identify functionally coupled regions followed by repeated measures ANOVAs of the factor scores. In each ANOVA, the effect of N_2O on the $rCMR_{glu}$ (in per cent of mean) was tested. *Post hoc* testing with paired Student's *t*-test (two-tailed) was performed when the ANOVA indicated significant effects in order to clarify which regional effect mainly contributed to the significance.

The relative change in distribution was calculated as the differences between the relative $rCMR_{glu}$ values for measurements, with and without N_2O.

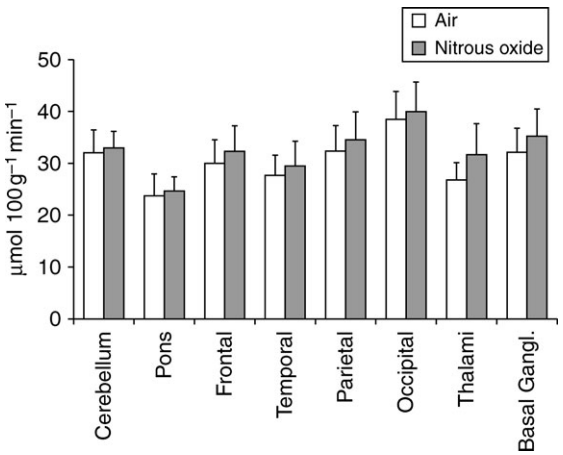

Fig 2 Regional CMR_{glu} in the brain through the different regions during inhalation of N_2O 50% (in O_2 30% in N_2), and O_2 30% in N_2. The results are presented as mean values (SD) (n=7).

Physiological data were analysed by two factor repeated measures ANOVA with correction for departure from sphericity for the interaction data.[18] Significant ANOVA interactions were further regionally analysed with Student's paired *t*-test.

$P \leq 0.05$ was considered statistically significant. All values are given as mean (SD). All statistical analyses were performed using the Stat View program version 5.0.1 for Windows (SAS Institute, Cary, NC, USA).

Results

Physiological values for inhalation of oxygen-enriched air and for inhalation of N_2O 50% is presented in Table 1. The mean haemoglobin value was 140 g $litre^{-1}$, range 120–154 g $litre^{-1}$.

All volunteers experienced psychogenic changes in cerebral function during N_2O inhalation with reduction in wakefulness, vision, hearing, and touch, whereas there was an increase in impulsiveness.

The volunteers had a global CMR_{glu} of 27 (3) μmol 100 g^{-1} min^{-1} when breathing oxygen-enriched air. Inhalation of N_2O 50% did not change global CMR_{glu} 30 (5) μmol 100 g^{-1} min^{-1} significantly, whereas it induced a significant change in the distribution (F=7.7, P<0.0001) (Fig. 2, Table 2). PCA analysis identified the factors explaining 89% of the variance in the ROIs. These were high negative loading in frontal, parietal, and temporal cortex *vs* positive loading in pons; high negative loading in cerebellum and positive in thalamus as well and basal ganglia; and finally high negative loading in the occipital region. Only high negative loading in cerebellum and positive in thalamus as well and basal ganglia reached the level of significance (P<0.0001).

Comparing the individual ROIs, N_2O significantly increased metabolism in thalamus by 22 (23%) (t=2.59, P<0.05) and basal ganglia by 14 (17)% (t=2.20, P<0.05) (Fig. 3).

In the different ROIs, inhalation of N_2O 50% changed the metabolism as shown in Figure 3. Representative PET and SPECT scans (CBF) under the same conditions with and without N_2O are presented in Figure 4.

There was a significant difference of the results whether N_2O or oxygen-enriched air was inhaled first (F=5.48, P<0.0001). In the three volunteers who started with N_2O

Table 1 Physiological variables during the two experimental conditions. Values are presented as mean (SD). Sa_{O_2}, peripheral oxygen saturation. *P<0.05

	O_2 30%	N_2O 50%
n	10	10
End-tidal CO_2 (kPa)	5.0 (0.3)	4.5 (0.4)*
Sa_{O_2} (%)	100 (0.3)	100 (0.3)
MAP (mm Hg)	87 (11)	92 (12)
Heart rate (beats min^{-1})	64 (8)	66 (14)

Table 2 Global and regional cerebral metabolism (CMR_{glu}) (μg 100 g^{-1} min^{-1}) in different experimental conditions. PET measurement (inhalation of O_2, before N_2O, and inhalation of N_2O, after O_2) represents the main study with inhalation of O_2 followed by N_2O. The following two conditions (inhalation of N_2O, before O_2, and inhalation of O_2, after N_2O) were the investigations starting with N_2O inhalation and where a lingering affect of N_2O was found. Values are presented as mean (SD)

PET measurement	Inhalation of O_2, before N_2O	Inhalation of N_2O, after O_2	Inhalation of N_2O, before O_2	Inhalation of O_2, after N_2O
Global	26.8 (3.0)	30.1 (5.0)	30.5 (4.1)	32.3 (1.8)
Region				
Cerebellum	31.0 (5.2)	33.3 (3.4)	31.6 (3.6)	34.3 (0.9)
Pons	23.8 (4.8)	25.1 (2.9)	23.2 (3.1)	24.3 (2.4)
Frontal	28.2 (3.3)	31.8 (5.2)	33.4 (5.0)	34.8 (2.7)
Temporal	25.7 (2.7)	29.3 (5.4)	29.6 (4.6)	32.2 (2.9)
Parietal	30.1 (3.6)	33.8 (5.4)	36.7 (5.8)	37.8 (3.0)
Occipital	36.7 (5.4)	40.4 (6.6)	39.0 (4.4)	43.2 (1.3)
Thalami	27.0 (3.6)	32.9 (6.1)	28.9 (5.1)	26.7 (2.3)
Basal ganglia	31.4 (4.9)	35.9 (5.5)	33.5 (4.9)	34.5 (1.7)
No. of subjects	7	7	3	3

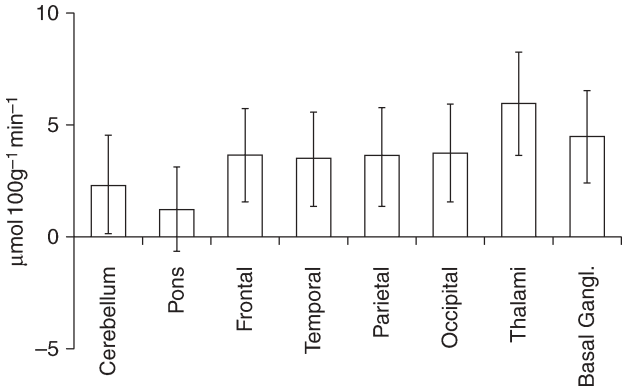

Fig 3 The change in regional CMR_{glu} due to inhalation of N_2O 50%. The results are presented in absolute values and as mean values (SD) (n=7).

inhalation, the CMR_{glu} was 30 (4) μmol 100 g^{-1} min^{-1} during N_2O inhalation and 32 (2) μmol 100 g^{-1} min^{-1} when air was inhaled. The effects on the different ROIs are presented in Figure 5.

Discussion

During inhalation of oxygen-enriched air, the global CMR_{glu} of the human brain was 27 μmol 100 g^{-1} min^{-1} that is in accordance with former similar studies.[14] In the present study, inhalation of N_2O 50% in healthy young men resulted in only a minor and non-significant increase in global CMR_{glu}. This is in fact not a surprising finding as no human study has demonstrated any increase in CMR due to N_2O inhalation; previous studies were limited by the fact that another anaesthetic drug was present during the investigation[15 16] and which has been used to explain the lack of effect of N_2O on CMR.[17] In the study by Kaisti and colleagues,[16] where adjunct of N_2O counteracts the $CMRO_2$ and CBF reduction by propofol and

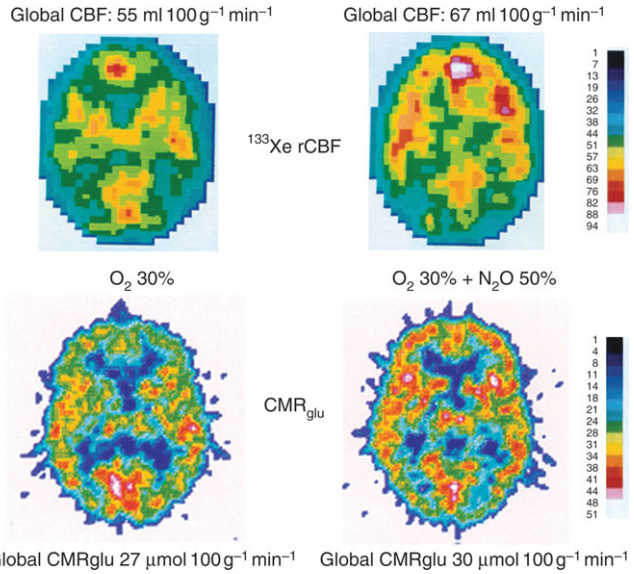

Fig 4 Representative pictures from scans in one of the volunteers during inhalation of oxygen-enriched air (30% O_2) (left pictures) and during inhalation of nitrous oxide (50% N_2O) (right pictures). The top figures represent three-dimensional CBF measurements with ^{133}Xe and SPECT from a former study[10] and the bottom figures, the cerebral metabolism (CMR_{glu}), from this study, in concomitant situations.

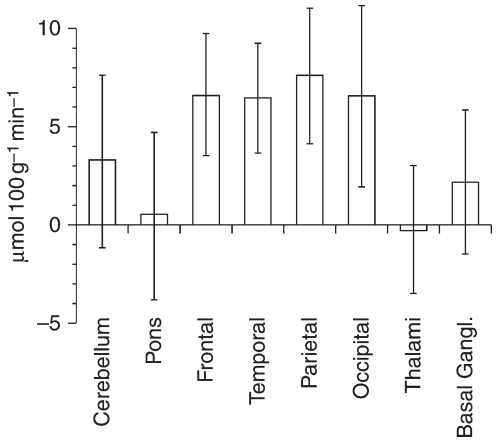

Fig 5 Figure demonstrating the lingering effect on the regional CMR_{glu} when N_2O (N_2O 50%, O_2 30%) was inhaled first followed by oxygen-enriched air (O_2 30%) (n=3).

sevoflurane, the amounts of propofol and sevoflurane given is lowered when N_2O was added, in order to keep the anaesthetic level constant. The effect could be due to a reduction in the basic anaesthesia or an increase in CMR due to the N_2O addition. In support of this assumption, N_2O generally increased the CMR in animals if administered alone,[7] but this increase in metabolism could be reduced or abolished by augmenting the anaesthetic level through addition of other anaesthetics. The animal studies, together with the fact that N_2O changes the EEG pattern in humans,[15 18 19] have been used to postulate that N_2O

increases CMR also in man, which our study does not support.

Compared with inhalation of oxygen-enriched air, N_2O 50% inhalation made the participants hyperventilate with a significant lowering of the E'_{CO_2} by 0.5 kPa (4 mm Hg), which is in accordance with other studies.[4 5] It is generally accepted that changes in CO_2 affect the CBF, but not CMR which remains unaffected of CO_2 changes within arterial CO_2 levels of 2–10 kPa (15–80 mm Hg).[20] One possible reason for the reduced effect of N_2O on the CMR_{glu}, as suggested by Stapleton and colleagues,[21] can be explained by an adaptation to lower anxiety during the second PET scan. However, most of the participants in the present study had also participated in a former study evaluating the effect of N_2O on the rCBF,[6] which would be expected to relieve the anxiety effect of these participants. Furthermore, N_2O 50% inhalation has profound psychogenic effects,[4–6] making it difficult to extrapolate the findings of Stapleton and colleagues[21] to our experimental condition. We also noted that participants who started with inhalation of N_2O 50% tended to increase global CMR_{glu} during the succeeding inhalation of oxygen-enriched air. As volunteers inhaling air twice did not change their metabolism, this observation is best explained by residual effects of N_2O on the succeeding CMR_{glu}, and does not favour the view that CMR should generally be decreased at the second measurement.

Inhalation of N_2O 50% has been reported to increase global CBF by 22–40% in man.[4–6] This effect is mainly believed to be due to the mental activation by N_2O as changes in rCBF should normally be directly coupled to changes in rCMR.[22] In this study, inhalation of N_2O 50% induced a non-significant increase in the global CMR_{glu} of 10% (Fig. 4). This contrasts to the earlier findings where CBF increased by 22–40%, indicating that there might be a direct or indirect effect on the cerebral arteries in addition to the cerebral metabolic effect. In support of this theory, addition of N_2O to an isoflurane anaesthesia during normocapnia resulted in an increased CBF without any influence on the rCBF pattern created by isoflurane.[23] This uncoupling between CBF and CMR has not been found to be due to a direct effect on the human cerebral vessels by N_2O,[6] opening up for alternative explanations to this discrepancy.

Inhalation of N_2O altered the regional distribution of CMR_{glu}, which increased in thalamus and basal ganglia. Utilizing the same technique to identify ROIs, N_2O inhalation was found to augment rCBF in most brain regions, although thalamus and basal ganglia were among the regions with the greatest change.[6] Even though we do not find a complete match between flow and metabolism, such conformity has been verified for inhalation of N_2O 20%.[24] The correlation between flow and metabolism may therefore explain the regional cerebral flow pattern found during N_2O inhalation,[6] which, however, is generally increased (uncoupled) due to the higher concentration of N_2O inhaled. The flow pattern gave the impression that N_2O inhalation increased flow through regions anatomically associated with the limbic system.[25] In support of the theory of limbic system activation by N_2O, we have previously reported[6] that N_2O stimulated thoughts and emotions, which normally are dealt with by the limbic system. In addition, former reports have shown an increased EEG activity in the limbic regions in cats[26] and in humans[27 28] during N_2O inhalation.

In conclusion, inhalation of N_2O 50% resulted in a small and non-significant increase in global CMR_{glu}. This effect on the global metabolism was mainly attributed to an increased metabolism in thalamus and basal ganglia. The metabolic effect of N_2O may linger in the brain more than 1 h after clinical recovery from anaesthesia.

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