Possible mechanism behind the vasodilating effect of nitrous oxide in the human brain
P Reinstrup, K Hesselgard, R Ekman

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Abstract

Background: Nitrous oxide (N₂O) increases CBF in humans but does not influence the tone in isolated human cerebral arteries. It has a relatively small effect on human global cerebral metabolism. Hence, the effect of N₂O is not secondary to an increase in cerebral metabolism.

Methods: In the present study we measured the plasma concentrations of the cyclic nucleotides cAMP and cGMP as well as two vasodilator peptides (VIP and CGRP) in healthy volunteers before and during inhalation of 50% N₂O.

Results and conclusion: N₂O inhalation elevated the plasma concentration of cGMP from 7.06± 0.81 to 8.03± 1.01 pmol/l (p=0.013) but failed to influence the plasma levels of cAMP, VIP and CGRP. This suggests that N₂O mediates at least part of its vasodilating effect through an increase in cGMP.

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INTRODUCTION
Nitrous oxide (N₂O) is a widely used anaesthetic that previously was considered to be relatively inert on the cerebral circulation. However, it has been reported that N₂O may have striking effects on the cerebral blood flow (CBF) in humans. CBF was increased by almost 50% in male volunteers, suggesting that N₂O may be hazardous in situations with increased intracranial pressure or a decreased cerebral elastance, situations that are not at all uncommon in neuroanaesthesiological practice.

Considering the pronounced effect on CBF, it was surprising that N₂O was totally inactive on isolated human cerebral arteries. Hence, the increase in blood flow elicited by N₂O must be secondary, for instance to an elevated cerebral metabolic rate (CMR), or to a release of dilatory mediators. Due to the observation that N₂O does not increase CMR globally in humans to the same extent as CBF, the latter of these assumptions seem most probable.

The present study was designed to identify the mediators of the N₂O-induced vasodilatation. We have measured the dilatory second messengers cyclic Guanosine monophosphate (cGMP) and cyclic adenosine monophosphate cAMP as well as vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) in plasma from healthy males exposed to N₂O.

METHODS
Ten male volunteers (aged 25 to 40 years) participating in a study on the effect of N₂O on cerebral glucose metabolism (PET after ³¹F-deoxyglucose, Reinstrup et al.) were included in the study. The ethics committee for human studies approved the protocol and written informed consent was obtained from each participant.

EXPERIMENTAL PROCEDURE
The participants were breathing spontaneously into a facemask held in place by rubber bands. Oxygen enriched air (total O₂ content 30%) was administered and a blood sample (20 ml) was withdrawn from the cubital vein after 35
minutes. Following this, they were exposed to a gas mixture consisting of 20% N2, 30% O2 and 50% N2O. After 35 minutes of exposure to N2O a second blood sample was obtained and the N2O administration was then discontinued. Heart rate and blood pressure were regularly checked during the experiment. The quantities of end tidal (Et) carbon dioxide (CO2), N2O and arterial haemoglobin O2 saturation were continuously monitored with an Ohmeda 4700 OxiCap (BOC Health Care, Louisville, KY, USA).

CHEMICAL ANALYSIS

Blood samples were collected in EDTA containing test tubes that were immediately immersed in ice-cold water. The samples were rapidly transported to the laboratory, centrifuged at +4°C and 2000G for 10 minutes. The plasma was subsequently allotted in polypropylene tubes that were frozen at -20°C. The frozen samples were kept at -20°C, transported on dry ice and thawed immediately before analysis. The measurements of cAMP and cGMP were performed with RIA at the Department of Clinical Pharmacology in Lund. After a purification process, the amounts of cAMP and cGMP were quantified using 125I-cAMP and 125I-cGMP (RIA kits from RIANEN, Du Pont Co., Boston, MA). 3H-cAMP and 3H-cGMP were added in order to determine the recovery during the purification process. The mean recovery for cAMP and cGMP were 54% and 56%, respectively.

For the RIA of VIP and CGRP the samples were analysed (Dept of Neurochemistry, Sahlgrenska University Hospital, Malmö) in serial dilutions optimised to the linear part of the standard curve and corrected for non-specific binding. Immunoreactive CGRP was quantified using a rabbit antiserum in a final dilution of 1:37,500. This allows measurements of CGRP-like material with a minimum of 10 pmol/l. The interassay variation was <12%. Immunoreactive VIP was determined using a rabbit antiserum at a final dilution of 1:60,000. The detection limit was 6 pmol/l and the interassay variation was <8.5%.

CALCULATIONS AND STATISTICAL ANALYSIS

All data from the chemical analyses are presented as molar concentrations. The results are presented as mean values ± standard error of the mean (SE). Determinations of statistical difference between paired groups of data were performed with two tailed t-tests, preceded by analysis of variance (ANOVA) if multiple comparisons were performed when indicated.

RESULTS

Physiologic parameters of the two experimental situations are presented in table 1. There were no statistically significant differences between the situations except for a lowering of the EtCO2- values during inhalation of N2O (table 1).

Figure 1

Table 1: Physiologic values at the time of blood sampling during inhalation of oxygen-enriched air (O2) or nitrous oxide (N2O). The values are presented as mean ± SE, n=10. EtCO2= end tidal concentration of CO, SaO2= arterial O2 saturation, MABP= mean arterial blood pressure. NS= non significant

<table>
<thead>
<tr>
<th></th>
<th>O2</th>
<th>N2O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtCO2 (kPa)</td>
<td>5±1</td>
<td>4.3±0.6</td>
<td>0.0062</td>
</tr>
<tr>
<td>SaO2 (%)</td>
<td>100±0.1</td>
<td>100±0.2</td>
<td>NS</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>98±2</td>
<td>91±4</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate</td>
<td>59±2</td>
<td>60±4</td>
<td>NS</td>
</tr>
</tbody>
</table>

CYCLIC NUCLEOTIDES

Exposure to 50% N2O had no significant effect on the serum levels of cAMP (table 2). However, we found a significant increase in the levels of cGMP during N2O inhalation (table 2).

Figure 2

Table 2: Concentrations (pmol/l) of cyclic nucleotides (mean ± SE, n=10). NS= non significant

<table>
<thead>
<tr>
<th></th>
<th>O2</th>
<th>N2O</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>20.3±1.5</td>
<td>24.0±3.4</td>
<td>NS</td>
</tr>
<tr>
<td>cGMP</td>
<td>7.06±0.81</td>
<td>8.03±1.01</td>
<td>0.013</td>
</tr>
</tbody>
</table>

PEPTIDES

The concentrations of both peptides were not infrequently below the detection level, which complicated the interpretation of the data. Measurements on VIP had to be excluded for 3 participants because the concentrations of VIP were below the level of detection during both experimental conditions. The same was the case with CGRP concentrations in 4 subjects. Of the remaining volunteers VIP levels increased in 3, were unchanged in 2 and decreased in the remaining 2 during exposure to N2O (range <5-7 pmol/l with O2 and <5-8 with N2O). N2O caused the concentrations of CGRP to increase in 3, remain unchanged in 1 and decrease in 2 participants (range <10-28 pmol/l).
with \( O_2 \) and <10-26 with \( N_2O \). Furthermore, all values were within the limits for normal subjects. Hence, it can be concluded that \( N_2O \) influenced the concentrations of neither VIP nor CGRP.

**DISCUSSION**

In the present study we found that inhalation of 50% \( N_2O \) increased the plasma concentrations of cGMP in humans whereas the levels of cAMP, VIP and CGRP were unchanged. The increase of cGMP was not more than about 14%, but the plasma level only mirrors the intracellular concentration that should be considerably higher.

\( N_2O \) has been shown to augment CBF in humans and also to redistribute the flow towards central and frontal parts of the brain. We have recently observed that this effect on CBF is not correlated to a corresponding quantitative increase in the human brain metabolism, leaving the possibility that \( N_2O \) is a vasodilator. However, \( N_2O \) is inactive in isolated human cerebral arteries. Considering this observation, it is not surprising that the production of one of the intracellular second messages mediating vasodilatation is elevated. In fact, one would a priori expect one or another vasodilator to be increased.

The intracellular production of the cyclic nucleotide cGMP is an intermediate step mediating the hyper polarization by nitric oxide (NO) in vascular smooth muscle. This, in turn, causes the muscle to relax in response to NO. This substance, also known as endothelial relaxing factor (EDRF), mediates the endothelial dependent relaxation to several substances such as acetylcholine and substance P. Hence, these substances cause an increase in cGMP levels with NO production as an intermediate step. It has been demonstrated that a metabolic conversion between NO and \( N_2O \) can take place, implying that it is not necessarily so that \( N_2O \) stimulates the production of some vasodilator which in turn increases the production of NO. Instead one may speculate that \( N_2O \) can be directly converted into NO in the blood stream or elsewhere in the body. This hypothetical conversion obviously does not take place in the vessel walls since \( N_2O \) does not relax human cerebral arteries in vitro. Therefore, it seems less likely that \( N_2O \) stimulates the production of substances acting via endothelial release of NO.

Not all dilatory responses are mediated by cGMP. Many endogenous substances, such as dilatory prostaglandins \( \text{PG} \) and VIP, increase the production of cAMP. Presently the levels of cAMP did not rise during inhalation of \( N_2O \), suggesting that substances acting on adenylate cyclase did not mediate the dilatory effect, the enzyme producing cAMP. This is corroborated by our finding that the plasma concentration of VIP was unaffected by exposure to \( N_2O \). The implication of the findings with CGRP is less clear than with VIP since the concentrations of CGRP varied considerably between the participating individuals. This may possibly have concealed an effect of \( N_2O \) on the levels of this substance.

Since we have not studied the effect of \( N_2O \) on all possible vasodilators our findings cannot be taken as a conclusive proof that the effects are mediated entirely by cGMP. Other substances may stimulate guanylyl cyclase, one of them being CO\(_2\). During inhalation of \( N_2O \) the subjects hyperventilated resulting in hypocapnia that could be expected to reduce the cGMP production. This may explain the relatively low augmentation of cGMP-concentrations in the present study. We have previously observed that the increase in CBF during \( N_2O \) inhalation was reduced by half to 22\% if the subjects were hyperventilated, making it easier to correlate the effects on CBF and cGMP to each other. Thus, there are several indicators suggesting that it is unreasonable to postulate that \( N_2O \) increases the production of cGMP in the human body.

In summary, inhalation of \( N_2O \) increased the concentrations of cGMP in humans whereas the concentrations of cAMP, VIP and CGRP seemed to be unaffected. This observation suggests that the increase in CBF during \( N_2O \) inhalation is at least partly mediated by cGMP.

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**References**

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