Influence of equianaesthetic concentrations of nitrous oxide and isoflurane on regional cerebral blood flow, regional cerebral blood volume, and regional mean transit time in human volunteers

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Nitrous oxide and isoflurane have cerebral vasodilatory effects. The use of isoflurane in neuroanaesthesia is widely accepted, whereas the use of nitrous oxide in neuroanaesthesia is still the subject of debate. In the present study, contrast-enhanced magnetic resonance (MR) perfusion measurement was used to compare the effects of 0.4 MAC nitrous oxide ($n=9$) and 0.4 MAC isoflurane ($n=9$) on regional cerebral blood flow (rCBF), regional cerebral blood volume (rCBV) and regional mean transit time (rMTT) in spontaneously breathing human volunteers. Nitrous oxide increased rCBF and rCBV in supratentorial regions more than did isoflurane. Isoflurane, by contrast, increased rCBF and rCBV in basal ganglia more than did nitrous oxide. An increased rMTT was caused by a relatively greater increase in rCBV than in rCBF supratentorially by isoflurane and infratentorially by nitrous oxide. In conclusion, nitrous oxide increases rCBF and rCBV predominantly in supratentorial grey matter, whereas isoflurane increases rCBF and rCBV predominantly in infratentorial grey matter.

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Nitrous oxide has been used in anaesthesia for more than a century1 because of its dose-dependent analgesic and anaesthetic properties.2 The use of nitrous oxide in certain situations (e.g. intracranial surgery), however, is still the subject of debate as the drug is a potent cerebral vasodilator.3 In combination with potent inhalational anaesthetics, e.g. isoflurane, nitrous oxide is known to increase mean cerebral blood flow (CBF).4 By contrast, isoflurane, which has become the anaesthetic of choice for neuroanaesthesia,5 was shown to decrease global CBF in a dose-dependent manner in animals.6 A systematic study comparing nitrous oxide with isoflurane for their regional effects on CBF and simultaneously on regional cerebral blood volume (rCBV) in humans has not yet been conducted.

The present study used contrast-enhanced magnetic resonance imaging (MRI) perfusion measurements to investigate the influence of nitrous oxide and isoflurane on regional cerebral blood flow (rCBF), rCBV and regional mean transit time (rMTT). rMTT was used to analyse changes in rCBV in relation to changes in rCBF.

Methods

After approval by the local university ethics committee and written informed consent, 20 right-handed, non-smoking male volunteers (ASA physical status I) with no history of drug or alcohol abuse underwent MRI measurement of contrast-enhanced cerebral perfusion on two consecutive days. For each volunteer, the order of measurements (e.g. control and test-drug measurement), which were separated by 24 h, was allocated randomly. The volunteers were then allocated randomly to receive either isoflurane or nitrous oxide as the test drug.

Wearing a closely fitting face-mask, the volunteers breathed to achieve normocarbia [end-tidal carbon dioxide concentration ($\text{ETCO}_2$) 40 mm Hg, $F_{\text{IO}_2}$ 0.5] during the control measurement and administration of isoflurane [fraction of
expired isoflurane 0.45 (SD 0.02)%; 0.4 MAC)\(^7\) \((n=10)\) or nitrous oxide [fraction of expired nitrous oxide 45 (2)%; 0.4 MAC]\(^8\) \((n=10)\). A minimum of 10–15 min was allowed for stabilization of the end-tidal concentration of either anaesthetic before contrast-enhanced MRI perfusion measurement. The volunteers had been trained both by verbal instruction and by watching the capnographic trace of the monitor on the day before the MRI session. During the experiment, breathing at a constant \(V'\text{CO}_2\) (e.g. 40 mm Hg) was supported by voice command when necessary. The fraction of inspired and expired isoflurane, nitrous oxide, oxygen (\(F_{\text{I}O_2}\), \(F'E'_\text{CO}_2\), \(E'_\text{CO}_2\), respiration rate, non-invasive mean arterial blood pressure (MAP) and pulse oximetry haemoglobin saturation (\(S_p\text{O}_2\)) were monitored (S/5 MRI-Monitor™, Datex-Ohmeda, Helsinki, Finland). Quick Cal calibration gas (Ref. 755582; Datex) was used to calibrate the monitor.

MRI measurements were performed with a 1.5-tesla whole-body scanner (Magnetom Vision; Siemens, Erlangen, Germany) using a standard circular polarized head-coil. Single-shot echoplanar imaging (EPI) was performed with a repetition time of 2 s and an echo time of 64 ms. An acquisition matrix of 64\(\times\)128 (field of view 22\(\times\)22 cm, in-plane resolution 1.7\(\times\)3.4 mm) was used. The slice thickness was set to 5 mm (slice gap 1.25 mm) and 15 slices were measured simultaneously. A paramagnetic contrast agent gadolinium-diethylene triamine pentaacetic acid (Gd-DTPA, 0.1 mmol kg\(^{-1}\)) was injected into an antecubital vein at the rate of 9 ml s\(^{-1}\) using an MR-compatible power injector (Spectris; Medrad, Pittsburgh, PA, USA). EPI scans \((n=60)\) were performed at 2-s intervals to cover the entire passage of the contrast agent through the brain. Six scans (6/60 scans) taken before injection were used as the baseline.

\(r\text{CBV}\) and \(r\text{CBF}\) were calculated by a blinded investigator in regions of interest. In each subject, regions of interest were outlined freehand bilaterally in white and in grey matter (frontal, parietal, occipital, striatal and thalamic) on CBV maps (Fig. 1). Outlining regions of interest on corresponding anatomical \(T_2^*\)-weighted scans is not possible as EPI \(T_2^*\)-weighted contrast-enhanced perfusion scans have a known geometric distortion. In order to check the regions of interest for correct anatomical position, they were copied into the EPI \(T_2^*\)-weighted scans acquired before contrast media application. The definition of regions produced in this way is reliable and reproducible and is not biased to high signal areas on the CBV maps. Furthermore, varying partial volume effects as a result of the inclusion of white and grey matter in regions allocated primarily to one category, which could account for some of the differences between the regions, were minimized. Corresponding regions of interest contained similar numbers of pixels.

The basic concept used to determine CBV and CBF was described by Ostergaard \textit{et al.}\(^9\)–\(^11\) An improved gamma variate fit was used to reduce underestimation of the arterial input function, which otherwise leads to overestimation of \(r\text{CBV}\) and \(r\text{CBF}\) values.\(^12\)

After correction for the density of brain tissue,\(^13\) \(r\text{CBF}\) values are given in ml 100 g\(^{-1}\) min\(^{-1}\) and \(r\text{CBV}\) values in ml 100 g\(^{-1}\).

MTT, which defines the average time that any particle of tracer, e.g. contrast medium, remains within the region of interest,\(^14\) was calculated with the equation:

\[
\text{rMTT} = \frac{\text{rCBV}}{\text{rCBF}} \times 60
\]

Values for rMTT are given in seconds.
Statistical analysis

Data are presented as boxplots [e.g. median (upper, lower quartiles; range)]. Data were tested for normality with the Kolmogorov–Smirnov test. As the data were not normally distributed, within-group comparison was performed with a stable non-parametric test (e.g. the Mann–Whitney U-test), which also considered the similar, but not identical, numbers of pixels in comparable regions (e.g. unpaired test). A value of $P<0.05$ was considered statistically significant. Presentation of percentage changes in rCBF ($\Delta$-rCBF), percentage changes in rCBV ($\Delta$-rCBV) and percentage changes in rMTT ($\Delta$-rMTT) are merely descriptive. Differences between the agents in $\Delta$-rCBF, $\Delta$-rCBV and $\Delta$-rMTT were not tested for significance.

Results

All volunteers [$n=20$; age 26 (20–30) yr; weight 77 (SD 3) kg; height 179 (5) cm] completed the study without complication. Because of motion artefacts, measurements were not obtainable in volunteer no. 8 in the nitrous oxide group (1/10) and in volunteer no. 1 in the isoflurane group (1/10).

Responsiveness to verbal command, which was necessary once or twice in each volunteer in order to maintain normocapnia, was sustained during isoflurane ($n=9$) and nitrous oxide ($n=9$) administration. The contrast-enhanced perfusion measurement was commenced only after verbal command had produced stable normocapnia, so that no
further verbal stimulation was needed during perfusion measurement.

Haemodynamic (heart rate, MAP) and respiratory (SpO₂, EʻCO₂, respiratory rate) variables were influenced neither by isoflurane nor by nitrous oxide.

**rCBF**

Isoflurane increased occipital but not frontal or parietal grey matter rCBF (Fig. 2A), whereas nitrous oxide increased rCBF in all of these regions (Fig. 2B). In the basal ganglia, except for the right hemispheric striatum, however, isoflurane increased rCBF more than did nitrous oxide (Fig. 3A and B).

**rCBV**

Both isoflurane (Fig. 4A) and nitrous oxide (Fig. 4B) increased grey matter rCBV in practically all of the regions studied. In detail, nitrous oxide increased rCBV in the frontal and parietal grey matter more than did isoflurane. Right hemisphere occipital rCBV, however, was similarly increased by the two drugs. Both drugs increased striatal rCBV, whereas isoflurane increased thalamic rCBV more than did nitrous oxide (Fig. 5A and B).

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**Fig 3** rCBV in white matter (WM) and grey matter [striatal (GM_ST), thalamic (GM_TH), frontal (GM_FR), parietal (GM_PA), occipital (GM_OC)] during control and administration of isoflurane (0.4 MAC) (n=9) (A) and during control and administration of nitrous oxide (0.4 MAC) (n=9) (B). Data are presented as boxplots [e.g. median (upper, lower quartiles; range)]. *P<0.05.
Isoflurane (Fig. 6A) and nitrous oxide (Fig. 6B) increased grey matter rMTT in most regions. Isoflurane increased rMTT in the frontal, parietal and occipital grey matter more than did nitrous oxide, which decreased parietal rMTT. In contrast, in the basal ganglia nitrous oxide increased rMTT bilaterally more than did isoflurane (Fig. 7A and B).

**Discussion**

Nitrous oxide increased rCBF, rCBV and rMTT predominantly in supratentorial regions, whereas isoflurane produced predominantly infratentorial increases. In detail, isoflurane increased occipital grey matter rCBF, which has also been reported by Reinstrup et al. The concomitant unchanged frontal/parietal grey matter rCBF indicates a reversal of the anterior–posterior gradient in rCBF observed in normal resting-state studies. A similar reversal of the anterior–posterior gradient in rCBF was reported during coma and sleep. Therefore, the isoflurane-induced reversal of the anterior–posterior gradient in rCBF seen in the present study might well reflect an altered level of consciousness.

Like isoflurane, nitrous oxide increased occipital grey matter rCBF in our volunteers, but no reversal of the anterior–posterior gradient in rCBF was found, as the greatest effect of nitrous oxide on rCBF was found in the
frontal and parietal grey matter regions. A similar increase in frontal rCBF has also been reported for nitrous oxide.\textsuperscript{19}

It has been proposed that nitrous oxide causes disinhibition of frontal cortical activity,\textsuperscript{19} and this may prompt an exaggerated frontal distribution of rCBF, whereas isoflurane typically causes reversal of the anterior–posterior gradient in rCBF. Without psychological tests, however, it is not possible to establish a relationship between a drug-specific pattern of rCBF changes and a drug-specific altered level of consciousness in humans.

Fig 5 Percentage changes in rCBF in frontal, parietal, occipital grey matter (A) and in striatal and thalamic grey matter and in white matter (B) during administration of isoflurane (0.4 MAC) \((n=9)\) or nitrous oxide (0.4 MAC) \((n=9)\). Data are mean ± SEM.

Fig 6 Percentage changes in rCBFV in frontal, parietal, occipital grey matter (A) and in striatal and thalamic grey matter and in white matter (B) during administration of isoflurane (0.4 MAC) \((n=9)\) or nitrous oxide (0.4 MAC) \((n=9)\). Data are mean ± SEM.

In the infratentorial regions, isoflurane increased thalamic and striatal grey matter rCBF more than did nitrous oxide. A similar redistribution of rCBF to subcortical regions during isoflurane administration was reported in humans\textsuperscript{4} \textsuperscript{15} and animals.\textsuperscript{20} Although observed, the increase in rCBF in these regions was clearly less pronounced after nitrous oxide.

Nitrous oxide increased rCBV in frontal and parietal grey matter more but in infratentorial grey matter less than did isoflurane. These heterogeneous increases in rCBV during
administration of nitrous oxide and isoflurane demonstrate that these drugs are not simple vasodilators like hypercapnia, which causes a more uniform increase in rCBV.21

To further analyse changes in rCBF and rCBV, we used rMTT. rMTT defines the average time needed by a tracer to transit the region of interest.12 Because rMTT equals the ratio of rCBV to rCBF, any increase in rMTT (e.g. in the present study during nitrous oxide and isoflurane administration) reflects a relatively greater increase in rCBV than in rCBF. Isoflurane increased supratentorial rMTT more than did nitrous oxide, thereby indicating a greater increase in rCBV than in rCBF. In contrast, the infratentorial increase in rMTT was more pronounced under nitrous oxide. The extent to which drug-specific changes in rCBF and rCBV, as seen in the present study, are caused by direct vasodilatation and/or metabolically mediated effects on cerebral haemodynamics awaits further investigation. In this regard, it is a limitation of the present study that, during administration of nitrous oxide and isoflurane, metabolic data (obtained, for example, by means of phosphate spectroscopy), were not obtainable with the whole-body MR scanner we used (Magnetom Vision).

In conclusion, we have shown that nitrous oxide specifically increases rCBF and rCBV in supratentorial grey matter, whereas isoflurane specifically increases rCBF and rCBV in infratentorial grey matter.

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