EFFECTS OF NITROUS OXIDE AND VOLATILE ANAESTHETICS ON CEREBRAL BLOOD FLOW

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Nitrous oxide is a common component of neurosurgical anaesthesia, partly because it facilitates rapid emergence from anaesthesia [1] and partly because nitrous oxide is often thought to have modest effects on the brain [2, 3]. Volatile anaesthetics are known to increase cerebral blood flow (CBF) and, potentially, intracranial pressure (ICP) [4-6] and, to minimize this risk, many clinicians use a combination of nitrous oxide and volatile agent, thereby reducing the required concentration of the volatile drug. However, some studies have shown that the effects of nitrous oxide on CBF are substantial [7-14] and that it may also have important effects on ICP [15, 16].

We have compared the relative effects on CBF of increasing the concentration of volatile agent from 0.5 to 1.0 MAC with an equivalent anaesthetic dose (0.5 MAC) of nitrous oxide, utilizing autoradiographic methods in the rat.

MATERIALS AND METHODS

This study was approved by our institutional Animal Care and Use Committee. Fifty-two male Sprague-Dawley rats weighing between 310 and 390 g were anaesthetized with either 1.5% halothane or 2.0% isoflurane allocated randomly and 33% oxygen in nitrogen. Tracheotomy was performed and the lungs ventilated mechanically with a tidal volume of 2.5 ml at a rate of 60 b.p.m. Catheters were inserted into a femoral artery and vein via surgical incisions under local anaesthesia with 2.0% lignocaine. The arterial catheter was connected to a transducer for continuous measurement of arterial pressure and the venous catheter was used for administration of drugs and fluids. Heparin 200 iu and tubocurarine 2 mg were administered i.v. to each rat. A surgical preparation interval of 35 min (measured from the start of induction) was allowed.

Following surgical preparation, rats were allocated to one of six groups: two to receive 0.5 and four to receive 1.0 MAC anaesthesia. For the 0.5 MAC groups, halothane or isoflurane concentrations (delivered) were reduced to 0.55% and 0.70%, respectively. In two of the 1.0 MAC groups, rats received 1.10% halothane or 1.40% isoflurane and 70% nitrogen in oxygen, and in the remaining two 1.0 MAC groups they received the 0.5 MAC equivalent of either volatile agent
N₂O, VOLETILE ANAESTHETICS AND CBF

291

+70% nitrous oxide in oxygen. In all cases, the volatile agent administered remained the same as that used for surgical preparation. MAC values in the Sprague-Dawley rat for halothane and isoflurane were taken from White, Johnston and Eger [17] and for nitrous oxide (140%) from Difazio and colleagues [18]. After the anaesthetic regimens had been established, animals remained anaesthetized at steady-state delivered concentrations for an additional 55 min. Anaesthetic concentrations were monitored by a Puritan Bennett Model 222 anaesthetic agent monitor (Wilmington, Mass.) sampling gas from the expiratory limb of the anaesthetic system. Previous work in our laboratory has indicated that this technique reflects end-tidal concentrations with an accuracy of approximately 97% after 55 min of exposure to 1.0 MAC delivered volatile agent [19].

During this equilibrium period, the ventilator was adjusted to maintain normoxia (\( P_{\text{ao}_2} = 14.6-18.6 \text{kPa} \)) and normocapnia (\( P_{\text{co}_2} = 5.1-5.6 \text{kPa} \)). Arterial blood was analysed at 15-min intervals with an automated blood-gas analyser (Radiometer ABL-2, Copenhagen, Denmark). Body temperature was monitored via a rectal probe and maintained within the range 36.5-37.5 °C by surface heating or cooling. Mean arterial pressure (MAP) was monitored continuously and maintained within the range 90-100 mm Hg by infusion of donor whole rat blood as required.

CBF determination

Following the 55 min exposure to respective anaesthetic agent regimens, animals were given 1 ml of donor blood to compensate for subsequent blood loss during arterial sampling. Final MAP and arterial blood-gas values were recorded, and \(^{14}\text{C}-\text{IAP} \) and \(^{14}\text{C}\)-labelled iodoantipyrine (\(^{14}\text{C}-\text{IAP} \), specific activity 60 mCi mmol\(^{-1} \), New England Nuclear, Boston, Mass.) 75 \( \mu\text{Ci} \) kg\(^{-1} \) was infused i.v. at a constant rate over 45 s via an infusion pump. During this time, 10 discontinuous 20-\( \mu\)l arterial blood samples were collected for later determination of arterial isotope activity. Immediately upon completion of isotope infusion, animals were decapitated, the brains removed rapidly, and frozen in 2-methyl butane (−40 °C). If brain removal required more than 2 min, the brain was discarded. Timed arterial blood samples were placed separately on chromatography paper, dried for 24 h, and eluted for an additional 24 h in a mixture of water 1 ml and liquid scintillation mixture 9 ml (Beckman, Fullerton, CA.). Radioactivity was determined by liquid scintillation counting using an external quench correction.

Autoradiography and image analysis

Frozen brains were cut in 20-\( \mu\)m thick serial coronal sections with a cryostat at −20 °C. Quadruplicate sections taken at 240-\( \mu\)m intervals were mounted on glass slides, dried for 5 min on a 50 °C hot plate, and exposed to Kodak SB-5 autoradiographic film for seven days in an x-ray cassette with seven \(^{14}\text{C}\)-methylmethacrylate standards (Amersham, Arlington Hts, Ill.). Images of eight brain sections were chosen from each animal for further analysis based upon standardized anatomical landmarks over the rostral-caudal axis [19]. These autoradiographic images were converted to digitized optical density images on a scanning microdensitometer system (Eikonix-Kodak, Bedford, Mass.) with a camera aperture of 100 \mu\)m and a fixed focal length. Optical densities from autoradiographic images, standard radioactivity values in tissue equivalents and timed arterial blood radioactivity values were entered on a computer system. For optical density recalculation from image grey levels to CBF values, we utilized the equations developed by Reivich and colleagues [20] and modified by Sakurada and co-workers [21]; mathematical operations were performed using the National Institutes of Health cerebral blood flow program (C. Patlak, Bethesda, Maryland).

Data analysis

Individual digitized CBF images were pseudo-colour enhanced and displayed on a cathode ray screen. By an operator-controlled cursor, regions of interest were outlined and mean CBF was determined within each region, together with the area of that region in pixel units. Three primary regions of interest were hemisphere, neocortex, and subcortex. Olfactory and entorhinal cortices are limbic structures and were included in subcortex [22]. Ventricular spaces were subtracted before performance of CBF calculations.

For each section level within each animal, area-weighted hemispheric, neocortical and subcortical blood flow values were determined. These values were averaged over all eight sections evaluated, yielding mean values for each region of interest.
TABLE I. Physiological values (mean (SD)) measured immediately before the determination of CBF as a function of anaesthetic regimen. No significant differences were observed between groups

<table>
<thead>
<tr>
<th>Group</th>
<th>MAC</th>
<th>Total PaO₂ (kPa)</th>
<th>PaCO₂ (kPa)</th>
<th>Arterial ph</th>
<th>MAP (mm Hg)</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.5</td>
<td>5.2 (0.2)</td>
<td>16.4 (2.5)</td>
<td>7.38 (0.03)</td>
<td>97 (5)</td>
<td>36.9 (0.18)</td>
</tr>
<tr>
<td>B</td>
<td>0.5</td>
<td>5.3 (0.3)</td>
<td>15.7 (2.3)</td>
<td>7.38 (0.04)</td>
<td>97 (4)</td>
<td>37.0 (0.16)</td>
</tr>
<tr>
<td>C</td>
<td>1.0</td>
<td>5.3 (0.3)</td>
<td>17.8 (4.3)</td>
<td>7.38 (0.04)</td>
<td>95 (4)</td>
<td>36.9 (0.15)</td>
</tr>
<tr>
<td>D</td>
<td>1.0</td>
<td>5.2 (0.2)</td>
<td>14.6 (2.8)</td>
<td>7.37 (0.04)</td>
<td>96 (4)</td>
<td>37.0 (0.17)</td>
</tr>
<tr>
<td>E</td>
<td>0.5</td>
<td>5.2 (0.3)</td>
<td>16.1 (2.9)</td>
<td>7.38 (0.03)</td>
<td>94 (5)</td>
<td>36.9 (0.15)</td>
</tr>
<tr>
<td>F</td>
<td>0.5</td>
<td>5.3 (0.2)</td>
<td>17.0 (3.3)</td>
<td>7.38 (0.04)</td>
<td>94 (4)</td>
<td>37.0 (0.17)</td>
</tr>
</tbody>
</table>

Physiological values measured in all groups (hemisphere, neocortex and subcortex). Mean CBF values for each region of interest were compared between the six anaesthetic groups by one-way analysis of variance. Specific intergroup differences were identified using a Newman-Keuls test for multiple comparisons where indicated by a significant F ratio.

Physiological values were compared between anaesthetic groups by one-way analysis of variance. One animal from the 1 MAC isoflurane group was excluded from analysis because of PaCO₂ values deviating by more than two standard deviations from the group mean. One animal from the 0.5 MAC isoflurane group was excluded because of a brain removal time exceeding 2 min. Values throughout are given as mean (SD) with significance assumed when P < 0.05.

RESULTS

No significant differences were noted between anaesthetic groups with respect to baseline PaO₂, PaCO₂, MAP or body temperature values (table I).

Hemispheric blood flow (area-weighted mean value of eight rostral-caudal coronal sections) was similar for halothane and isoflurane at both 0.5 and 1.0 MAC concentrations (table II; fig. 1). For each agent, a significantly greater flow value was observed at 1.0 MAC compared with 0.5 MAC (P < 0.05). The addition of 70% nitrous oxide (0.5 MAC) to 0.5 MAC isoflurane resulted in hemispheric flow values greater than those observed at either 0.5 or 1.0 MAC isoflurane alone (P < 0.01).

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The combination of isoflurane + nitrous oxide also yielded hemispheric flow values greater than any of the groups receiving halothane or halothane plus nitrous oxide \( (P < 0.05) \). In contrast, while the combination of 70% nitrous oxide and 0.5 MAC halothane resulted in hemispheric flow values greater than those observed at 0.5 MAC halothane alone, the flow values observed in this group were not different from those receiving 1 MAC halothane alone.

In the neocortex, a different pattern emerged. At 0.5 MAC, cortical blood flow was greater in rats receiving halothane compared with those given isoflurane \( (P < 0.01) \). The distinction between the cortical CBF effects of these two agents was preserved at 1.0 MAC. For both agents, neocortical flow was increased when the anaesthetic concentration was increased from 0.5 to 1.0 MAC \( (P < 0.05) \). When nitrous oxide was added to 0.5 MAC of either volatile agent, cortical flow was significantly greater than values observed at 0.5 or 1.0 MAC for each agent alone (table II).

In the subcortex, the pattern matched more closely that observed in the hemisphere as a whole. Flow values at 0.5 and 1.0 MAC were similar between volatile anaesthetic agents at each depth of anaesthesia. The addition of nitrous oxide again significantly increased flow relative to 0.5 MAC values for each volatile agent, but only in the case of isoflurane did the additive effect of nitrous oxide + 0.5 MAC isoflurane cause flow to be greater than 1 MAC volatile anaesthetic alone.

**DISCUSSION**

The purpose of this investigation was to determine the relative magnitude of CBF response when an increase in depth of anaesthesia was achieved with either volatile anaesthetic alone or by the addition of an equivalent anaesthetic dose of nitrous oxide. Our results indicate that, for both regional and global CBF, there was an increase following the addition of nitrous oxide which was as great as that which occurred if a similar anaesthetic depth had been achieved with volatile agent alone. In most instances, the addition of 70% nitrous oxide resulted in greater CBF than did the addition of 0.5 MAC volatile agent. This was particularly true for isoflurane.

Several other studies have investigated the interactive effects of nitrous oxide and volatile anaesthetics on cerebral blood flow. Using the radioactive microsphere technique in swine, Manohar and Parks [9, 10] observed that the addition of 0.5 MAC nitrous oxide to 0.5 MAC halothane or isoflurane resulted in a greater blood flow than did a 1 MAC dose of either agent alone. The interpretation of these results was difficult, however, because of marked variations in mean arterial pressure (MAP) occurring between anaesthetic groups: MAP was significantly greater in those animals receiving nitrous oxide. Although cerebral autoregulation would be expected to be largely intact, and correcting for this discrepancy at 1.0 MAC anaesthesia [23], this has not been
evaluated either in swine or under the combined influence of nitrous oxide and volatile agent. Thus in the present study, we have kept MAP constant in all groups and we observed that the CBF effects of nitrous oxide were as great as those associated with a similar dose of halothane or isoflurane. Our data are therefore consistent with the conclusions of Manohar and Parks [9, 10] that the combination of nitrous oxide with volatile agents caused a substantial increase in CBF. However, while Manohar and Parks found that the nitrous oxide effect was more pronounced in the presence of halothane than isoflurane, the converse was observed in our study. This may be a reflection of species differences or variations in methodology.

Drummond, Scheller and Todd [12] have also investigated the CBF effect of nitrous oxide during background anaesthesia with halothane or isoflurane in the rabbit. As was observed by Manohar and Parks [6, 7], the addition of nitrous oxide to 0.5 or 1.0 MAC of either agent resulted in increased CBF. Although Drummond and colleagues maintained constant MAP by i.v. infusion of angiotensin II, they used a hydrogen clearance technique for measurement of cortical CBF. This technique measures flow in a tissue volume of approximately 1 mm in diameter about the implanted platinum electrode [24], and it has been shown that conclusions drawn from anaesthetic effects on cerebral blood flow are dependent upon where CBF is measured in the brain. For example, while 1 MAC halothane and 1 MAC isoflurane have nearly identical effects on global CBF, halothane causes a greater increase in cortical CBF than isoflurane [19]. It is possible, therefore, that the findings of Drummond and colleagues may only apply to the cortex and not to the brain as a whole. However, our data failed to confirm this hypothesis. On both a global level (area-weighted mean of eight hemispheric values taken over the rostral-caudal extent of the brain) and a regional basis (cortex and subcortex), an increase in CBF was observed with the addition of nitrous oxide at least as great as that caused by doubling the volatile anaesthetic concentration.

Does this study have any clinical relevance for the patient with intracranial hypertension? It is well known that nitrous oxide has been used for a very large number of craniotomies with no apparent evidence of worsened neurological outcome, except in the notable case of pre-existing pneumocephalus. However, we know that administration of nitrous oxide (particularly in combination with volatile agents) may cause increases in CBF and intracranial pressure which may or may not be responsive to hyperventilation [13, 25]. This suggests that it may be reasonable, at present, to continue to use nitrous oxide during craniotomy, but clinical studies and our data suggest that, in some patients, it may be more appropriate to discontinue nitrous oxide before the use of volatile agent. In addition, its use may not be beneficial when operative conditions arise such as uncontrollable brain bulk.

The mechanism whereby nitrous oxide increases CBF may be direct vasodilatation, but other data suggest a more complex phenomenon. Nitrous oxide alone has little or no effect on either CBF or the cerebral metabolic rate for glucose utilization in the rat [3, 26] and it is unclear why its effects on CBF are so pronounced in the same species in the presence of a halogenated anaesthetic agent. We do know that effects of volatile anaesthetics on CBF are to a large extent mediated by their respective effects on cerebral metabolism [27]. We may postulate that, if this relationship (coupling of flow and metabolism) is perturbed by nitrous oxide, the resultant CBF might be increased. This would be consistent with our observation that the addition of nitrous oxide to 0.5 MAC isoflurane—a greater cerebral metabolic depressant than halothane—had more profound effects on CBF than the combination of nitrous oxide with halothane. This hypothesis remains untested, but warrants investigation.

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