Desflurane affords greater protection than halothane against focal cerebral ischaemia in the rat

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Background. We studied the potential neuroprotective effects of halothane and desflurane, compared with the awake state, on infarct size following 2 h of intraluminal middle cerebral artery occlusion (MCAo) and 22 h of reperfusion.

Methods. Male Sprague–Dawley rats were anaesthetized with desflurane or halothane, intubated, and mechanically ventilated. Mean arterial pressure (MAP), blood gases, and pH were controlled. Body temperature was maintained at 37.5–38°C. Animals were assigned to one of four groups according to the anaesthetic type (halothane or desflurane) and the duration of anaesthesia: `short-duration', during the preparation only; `long-duration', during both preparation and ischaemia. Twenty-four hours after MCAo, infarcts were visualized by staining with 2,3,5-triphenyltetrazolium chloride. Two additional groups of rats were subjected to the same protocol as that of long-duration halothane and long-duration desflurane with additional pericranial temperature measurements made.

Results. Physiological parameters were comparable between the groups but MAP was higher (P<0.0001) in the short-duration groups. In the short-duration groups, cerebral infarct volumes were not significantly different between anaesthetics (short-duration halothane: 288 (61) mm³, mean (SD); short-duration desflurane: 269 (71) mm³, P>0.56). Compared with the awake state (short-duration groups), halothane and desflurane significantly reduced infarct volumes (long-duration halothane: 199 (54) mm³, P<0.0047 vs short-duration halothane; long-duration desflurane: 121 (55) mm³, P<0.0001 vs short-duration desflurane). The mean infarct volume in the long-duration desflurane group was significantly lower than that in the long-duration halothane group (P<0.0053). Pericranial temperatures were similar in the desflurane and halothane long-duration groups (P>0.17).

Conclusions. In rats, desflurane-induced neuroprotection against focal cerebral ischaemia was greater than that conferred by halothane.

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In clinical practice, carotid endarterectomy, clipping of cerebral aneurysm, and cardiopulmonary bypass are known to represent a high risk of transient focal cerebral ischaemia. Thus, identifying pharmacological agents, for instance the anaesthetic agent, which could provide cerebral protection against ischaemia may be useful. Volatile anaesthetics have been shown to reduce or delay neuronal death induced by experimental ischaemia. Indeed, halothane, isoflurane, and sevoflurane reduce infarct volume in focal cerebral ischaemia in rats.1–3 The neuroprotection induced by
halothane is maintained when the pericranial temperature is controlled. At the present time, no study has been reported on the effects of desflurane on histopathology following focal cerebral ischaemia. Nevertheless, isoflurane and desflurane improve neurological outcome following transient global cerebral ischaemia in rats and desflurane has been shown to attenuate the decrease in tissue oxygenation during transient middle cerebral artery occlusion (MCAo) in humans, as compared with thiopental. The aim of this study was, therefore, to determine whether desflurane could reduce focal ischaemic injury and to compare such potential reduction with that afforded by halothane. In this study we examined four groups of rats with temporary MCAo (2 h) followed by reperfusion with either desflurane or halothane anaesthesia during the experimental preparation. For each anaesthetic, one group remained anaesthetized for the duration of MCAo, while the other recovered from the anaesthetic regime following MCAo. A complementary study was also performed to compare pericranial temperatures following ischaemia under halothane and desflurane anaesthesia.

Materials and methods
Adult male Sprague-Dawley rats weighing 315 (35) g (R. Janvier breeding center, Le Genest-St-Isle, France) were used in the present study. The animals had free access to food and water in an animal room at constant temperature (22 (1)°C), humidity (55 (10)%) and with reversed light cycle. Experimental procedures conformed to the guidelines proposed in the Guide for the Care and Use of Laboratory Animals, and to the appropriate European directives and French national legislation.

Animal preparation
Animals were randomly selected to be anaesthetized with halothane (n=18) or desflurane (n=18) always delivered in oxygen:nitrous oxide (30:70%). After tracheal intubation, the animals lungs were artificially ventilated (Harvard Apparatus 683, MA, USA). Throughout the preparation period (45 min), halothane and desflurane concentrations were maintained at 1.5–1.6 and 11–12%, respectively, corresponding to 2.0 MAC. A catheter was inserted into the right femoral artery for continuous monitoring of mean arterial pressure (MAP) and the periodic analysis of blood gases and pH (Ciba Corning M328, Essex, UK). The catheter was tunnelled subcutaneously and exteriorized at the neck for use in awake animals. The animals were maintained normothermic (37.5–38°C) using a feedback controlled heating blanket (Harvard Apparatus Limited, Edenbridge, UK) connected to a rectal probe.

Experimental protocol and design
MCAo was performed by insertion of an intraluminal filament (made of a terminal cylinder of melting glue, 2 mm long, diameter 0.38 mm, attached to a nylon thread, 0.22 mm in diameter) into the lumen of the right external carotid artery. The filament was advanced into the internal carotid artery, 9 mm after the outer table of the skull and then secured to the external carotid artery. In this manner, the terminal cylinder is reproducibly placed at the MCA origin. For each anaesthetic, the rats were then randomly assigned to one of two experimental groups, which differed by the duration of anaesthesia. Four experimental groups were studied (Fig. 1).

Fig 1 Experimental protocol. The preparation of each animal lasted approximately 45 min. MCAo was maintained for 2 h, followed by reperfusion. The following day (24 h), infarcts were revealed by TTC staining. Four groups were studied which differed according to the anaesthetic drug used (halothane or desflurane) and the duration of anaesthesia, as shown. All anaesthetics were delivered in oxygen:nitrous oxide (30:70%).
anaesthetized for about 10 min with the same anaesthetic as used previously to withdraw the filament. In all rats, the filament was then removed, wounds were sutured and anaesthesia was discontinued.

Twenty-two hours later, the animals were again anaesthetized with the same anaesthetic as used previously and perfused transcardially at 120 mm Hg with heparinized saline for 2 min followed by 2,3,5-triphenyltetrazolium chloride 2% (Sigma, Saint Quentin Fallavier, France) for 8 min. The brain was removed and fixed by immersion in a paraformaldehyde solution 4%. The brain was then cut in 1 mm slices using a purpose made matrix. The slices were digitized. Infarcted regions were delineated using the public domain ImageJ software. The volume of infarction was calculated by integration over the whole brain of the infarcted surfaces.

In additional experiments, pericranial temperature was measured continuously with a probe inserted between the skull and the temporal muscle in rats exposed to the same protocol as that of long-duration halothane (n=5) and long-duration desflurane (n=5) groups.

### Statistical analysis

Values are given as mean (SD). Data were analyzed by ANOVA using Statview® software (Abacus Concepts, Berkeley, CA, USA) as detailed in the results section followed when appropriate by a two-tailed Student’s t-test. P<0.05 or P<0.05/number of comparisons (Bonferroni correction) was accepted as significant.

### Results

#### Volume of infarction

In all animals, an infarct was observed which comprised both the cortex and the striatum (Fig. 2). Infarct volumes in the four groups are summarized in Figure 3. The infarct volumes were compared by a two-way ANOVA (anaesthetic, duration), showing that there was no significant interaction (P=0.16). However, the hypothesis of an interaction was not rejected as the power of the analysis was low (power <0.28). Student’s t-tests with Bonferroni correction were then performed. In the halothane groups, a significant reduction of infarct volume (31%; P<0.0047) was obtained in the long-duration halothane group (199 (54) mm$^3$) as compared with the short-duration halothane group (288 (61) mm$^3$). Similarly, for desflurane, a reduction of infarct volume (55%; P<0.0001) in the long-duration desflurane group as compared with the short-duration desflurane group was found (121 (55) and 269 (71) mm$^3$, respectively). In the short-duration groups, the mean infarct volume in the desflurane group was not significantly different from that in the halothane group (P>0.56). In the long-duration groups, the mean infarct volume in the desflurane group was significantly lower (39%) than that in the halothane group (P<0.0053).

#### Physiological parameters

Blood samples were obtained before ischaemia, and 45 and 90 min after MCAo. As detailed there was no difference between groups for $P_{aCO_2}$ at all times. For $P_{aO_2}$, pH, and rectal temperature (Temp) there was no difference between the groups before ischaemia, with small but significant changes during ischaemia.

$P_{aO_2}$, $P_{aCO_2}$, arterial pH, and Temp were analysed using a three-way repeated measures ANOVA (factors: anaesthetic, duration of anaesthesia, time as repeated factor). For $P_{aCO_2}$ and Temp, there were no significant interactions and no main effects, except for Temp for which a significant difference (0.2°C) between the short-duration and long-duration groups was obtained (P=0.0003). For $P_{aO_2}$ and pH, interactions with time were found (P<0.03), and data
obtained before and after MCAo were subsequently analysed separately. For these parameters, a three-way repeated measures ANOVA was performed on data obtained during ischaemia. No interaction ($P > 0.26$) with time was found so that values obtained after MCAo were averaged.

Next, two-way factorial ANOVA (anaesthetic, duration) was performed before and after MCAo. All data and statistics are summarized in Table 1. For $P_{aO_2}$ and pH, a significant effect of the duration factor on pH was obtained ($P < 0.02$). All other effects and interactions were not significant. After MCAo, no significant interaction was found for $P_{aO_2}$ ($P > 0.24$) but the factors precluded some significant effects (anaesthetic: $P = 0.0008$; duration: $P < 0.0001$). A significant interaction is obtained ($P < 0.0008$) for pH. Therefore, for each factor level, a Student’s $t$-test was performed. All comparisons were significant for pH ($P < 0.04$) and the largest difference between the groups was 0.07 pH units.

Before ischaemia there was a significant difference in MAP between halothane and desflurane groups. During ischaemia, there was a significant difference between the short-duration and long-duration groups at all times, but no effect of the anaesthetic (except for the first 20 min after MCAo for the short-duration groups). Detailed analysis of MAP is presented below.

Continuous MAP recordings were averaged over 20 min periods (Fig. 4). MAP was analysed by a three-way repeated measures ANOVA (anaesthetic, duration, time). A second order interaction was found ($P < 0.0001$) and hence a two-way factorial ANOVA was performed for each time. Before ischaemia, only the anaesthetic factor was significant effect ($P < 0.022$). For the first time period after MCAo, a significant interaction was obtained ($P < 0.0015$). For both anaesthetics, a significant effect was obtained for the duration factor ($P < 0.012$). There was also a significant difference between short-duration halothane and short-duration desflurane groups ($P = 0.001$). For all other times after MCAo, only the duration factor was significant ($P < 0.0001$).

For pericranial temperature, a two-way repeated measures ANOVA (factors: anaesthetic, time as repeated factor) was performed and no interaction was found ($P > 0.21$). The anaesthetic had no significant effect on pericranial temperature ($P > 0.18$) with an average difference of 0.12°C (Fig. 5). Physiological parameters (data not shown) were similar to those presented above.

### Discussion

The results of the present study indicate that halothane and desflurane induce neuroprotection against transient focal

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**Table 1** Physiological parameters before and during ischaemia. Values are means (SD); $P_{aO_2}$, arterial oxygen partial pressure; $P_{aCO_2}$, arterial carbon dioxide partial pressure; pH, arterial pH; Rectal Temp, rectal temperature. Data are analysed by ANOVA (see text for details). *P*<0.05 vs long-duration halothane group. *P*<0.05 vs long-duration desflurane group. *P*<0.05 vs long-duration halothane group and vs short-duration desflurane group. *P*<0.05 short-duration vs long-duration groups.

<table>
<thead>
<tr>
<th></th>
<th>$P_{aO_2}$ (mm Hg)</th>
<th>$P_{aCO_2}$ (mm Hg)</th>
<th>pH</th>
<th>Rectal Temp* (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before ischaemia</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Long-duration halothane</td>
<td>133 (12)</td>
<td>35.9 (2.0)</td>
<td>7.43 (0.05)</td>
<td>37.6 (0.4)</td>
</tr>
<tr>
<td>Long-duration desflurane</td>
<td>145 (30)</td>
<td>35.0 (2.2)</td>
<td>7.47 (0.02)</td>
<td>37.7 (0.1)</td>
</tr>
<tr>
<td>Short-duration halothane</td>
<td>129 (26)</td>
<td>36.1 (2.2)</td>
<td>7.41 (0.07)</td>
<td>37.8 (0.1)</td>
</tr>
<tr>
<td>Short-duration desflurane</td>
<td>131 (32)</td>
<td>35.4 (2.9)</td>
<td>7.42 (0.02)</td>
<td>37.8 (0.2)</td>
</tr>
<tr>
<td><strong>During ischaemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long-duration halothane</td>
<td>138 (18)</td>
<td>38.6 (3.7)</td>
<td>7.41 (0.06)</td>
<td>37.7 (0.1)</td>
</tr>
<tr>
<td>Long-duration desflurane</td>
<td>160 (26)</td>
<td>36.8 (3.0)</td>
<td>7.45 (0.03)</td>
<td>37.8 (0.1)</td>
</tr>
<tr>
<td>Short-duration halothane</td>
<td>83 (5)</td>
<td>37.0 (6.5)</td>
<td>7.47 (0.09)</td>
<td>37.9 (0.4)</td>
</tr>
<tr>
<td>Short-duration desflurane</td>
<td>94 (22)</td>
<td>38.7 (4.5)</td>
<td>7.41 (0.03)</td>
<td>38.1 (0.3)</td>
</tr>
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**Fig 4** Time course of MAP changes in the four groups. Continuous MAP recordings were averaged over 20 min periods. Two-way ANOVAs (duration, anaesthetic) were performed at each time period (following a three-way ANOVA, see text for details). *Significant main effect of the anaesthetic factor. *Significant difference between short-duration halothane and short-duration desflurane groups. *Significant effect of the duration factor at each time period.
cerebral ischaemia in rats, as shown by the measurement of infarct volumes 24 h after MCA occlusion. However, an eventual further increase in infarct volume at even longer times cannot be excluded. At the same MAC, halothane reduces infarct volume by approximately 30% and desflurane by approximately 50%.

Although clinical evidence is lacking, volatile anaesthetics have been shown to provide neuroprotective effects against ischaemia in various species and experimental models.\textsuperscript{1-5} It has been shown that 1.4 MAC of halothane or sevoflurane, as compared with the awake state, reduces infarct volume by approximately 72 and 57%, respectively, in rats exposed to 90 min of focal cerebral ischaemia.\textsuperscript{2} In the same model, 1.0 MAC of halothane remains neuroprotective even when brain temperature is maintained in normothermic conditions.\textsuperscript{3} Our results are in accordance with these findings in that the long-duration administration of halothane 1.1–1.2% during ischaemia in the present study provided a significant decrease in cerebral infarct volume in normothermic rats. Several experiments with isoflurane have shown that this compound (0.5, 1.0 MAC) protects the brain in rat models of global ischaemia\textsuperscript{4} but the protection against focal cerebral ischaemia is debatable. In comparison with nitrous oxide/fentanyl, isoflurane 2.5% reduces infarct volume and the frequency of transient ischaemic depolarizations in a 2 h MCAo rat model.\textsuperscript{1} It has been reported that 1.5 MAC isoflurane delays but does not prevent cerebral infarction in rats subjected to a 70 min focal ischaemia.\textsuperscript{10} Finally, in spontaneously hypertensive rats, infarct volume following 3 h of MCAo and 2 h of reperfusion in animals anaesthetized with isoflurane were larger than those in animals anaesthetized with halothane.\textsuperscript{11} Although desflurane improves neurological outcome following transient global cerebral ischaemia in rats\textsuperscript{4} and increases tissue oxygenation during transient MCAo in humans,\textsuperscript{2} no studies have been reported on the effects of desflurane on histopathology following focal ischaemia.

Physiological parameters are unlikely to be responsible for the differences in infarct size observed between long-duration and short-duration groups. Indeed, there was a significantly higher MAP in the short-duration groups as compared with the long-duration groups. As acute increases in arterial pressure decrease brain injury,\textsuperscript{12} one would expect smaller infarcts in the short-duration anaesthetized groups. The differences between groups concerning the other physiological parameters (i.e. $P_{aO_2}$, $P_{aCO_2}$, pH and rectal and pericranial temperatures) were minor and probably without any biological significance. The decrease in infarct volume in the long-duration groups may, therefore, be reasonably attributed to the inhalation of nitrous oxide and/or the volatile anaesthetics.

Nitrous oxide has been reported to have no effect or to increase cerebral ischaemic damage. In a 2-h rat reversible MCAo model, nitrous oxide 70% does not modify infarct volume.\textsuperscript{13} In a rat brain global ischaemia model, animals ventilated with nitrous oxide 70% and oxygen 30% (as compared with those ventilated with nitrogen 70% and oxygen 30%) had a worse outcome.\textsuperscript{9} Other authors have shown that nitrous oxide 50% impairs electrophysiologic recovery after 3.5 min of hypoxia in rat hippocampal slices.\textsuperscript{14} Therefore, in the present study, nitrous oxide is probably not responsible for the observed neuroprotection.

Volatile anaesthetics may decrease ischaemic damage by various mechanisms. First, volatile anaesthetics produce cerebral vasodilation. Halothane potently dilates intracerebral arterioles in the rat brain.\textsuperscript{15} In dogs, both isoflurane and sevoflurane significantly dilate pial arterioles, an effect mediated, at least in part, via activation of $K^+_{ATP}$ channels.\textsuperscript{16} In patients undergoing routine spinal surgery, sevoflurane and isoflurane produce a dose-dependent cerebral vasodilatory effect.\textsuperscript{17} Considering that vasodilation might produce an increase in intra-ischaemic cerebral blood flow and an increased supply of oxygen and glucose, vasodilation by volatile anaesthetics could be neuroprotective. Secondly, 0.75 MAC halothane or isoflurane reduces cerebral metabolic rate, an effect which accompanies an improved outcome from severe brain ischaemia in the rat.\textsuperscript{18} Thirdly, volatile anaesthetics produce hypothermia which is in itself neuroprotective.\textsuperscript{19,20} Such a mechanism does not apply to our present study as body temperature was controlled. However, we cannot rule out an effect on brain temperature.

Volatile anaesthetics have major effects on synaptic neurotransmission which may participate in their neuroprotective effects, given the well described deleterious effects of glutamate during ischaemia.\textsuperscript{21,22} There is increasing evidence that general anaesthetics enhance GABA-mediated synaptic inhibition\textsuperscript{23} and that they depress excitatory glutamate-mediated transmission.\textsuperscript{24} In rat hippocampal brain slices, electrophysiological studies have shown that volatile anaesthetics depress glutamate transmission.\textsuperscript{25} Volatile anaesthetics increase glutamate uptake as demonstrated in cultured astrocytes from the hippocampus of rat...
embryos exposed to enflurane, isoflurane, and sevoflurane. Furthermore, isoflurane reduces both L-glutamate and NMDA-mediated calcium influx in rat cortical brain slices. Volatile anaesthetics modulate the activity of GABA\(_A\) receptors by a direct action on the channel complex. The block of voltage-dependent Na\(^+\) channels by low concentrations of anaesthetics might have a significant role for neuroprotection because this attenuates membrane depolarization initiating the removal of the magnesium block of the NMDA receptor. Finally, halothane, enflurane, and isoflurane induce a dose-dependent uncoupling of gap junctions in primary cultures of mouse striatal astrocytes. This effect together with the effects on glutamate transmission, may partly explain why halothane inhibits spreading depression like-depolarizations following ischaemia, thus leading to a reduced infarct volume.

In our study, our study shows that desflurane reduces infarct volume following transient MCAo. Furthermore, this effect is greater than that induced by halothane. In our study, the concentration of halothane and desflurane during the preparation and focal cerebral ischaemia were equipotent. Therefore, the higher neuroprotection of desflurane cannot be attributed to the differences in the applied anaesthetic concentrations for the two agents.

In other respects, changes in pericranial temperature by volatile anaesthetics may influence infarct volume. When brain temperature is controlled, isoflurane 0.7% does not protect the rat brain from 75 min intraluminal MCAo. In contrast, the neuroprotection induced by halothane is maintained when pericranial temperature is controlled. Pericranial temperature correlates well with brain temperature. In our study, we find no significant difference in pericranial temperature under desflurane or halothane was found and the average difference was approximately 0.12°C. Therefore, in our study, the non-significant changes in brain temperature are probably not responsible for the higher neuroprotection conferred by desflurane.

The increase in brain tissue partial oxygen pressure and decrease in brain acidosis induced by desflurane may participate in its neuroprotective effect during MCAo. Although desflurane possesses most of the properties of the other volatile anaesthetics discussed above, it also induces a concentration-dependent biphasic effect on sympathetic activity. Indeed, with concentrations of desflurane under 6%, sympathetic activity is increased, while desflurane decreases sympathetic activity at higher concentrations. Thus, given the concentrations used in the present study, it may be assumed that desflurane reduces sympathetic activity and catecholamine release. In this respect, Engelhard and colleagues demonstrated that the neurological improvement induced by desflurane following global ischaemia was associated with a reduction in sympathetic activity and catecholamine release. Several studies have addressed the effects of catecholamines in cerebral ischaemia. In a rat model of incomplete ischaemia, ganglionic block decreases plasma catecholamine concentrations and improves neurologic outcome. In addition, neurologic outcome and stroke-related mortality were worse in rats with increased plasma epinephrine and norepinephrine concentrations compared with rats with ganglionic block. Moreover, pre-ischaemic depletion of brain norepinephrine decreases infarct size and improves neurologic outcome in normothermic rats. It is clear that sympathetic activity and adrenergic receptors modulate brain damage but the brain protection mechanisms suggested here against cerebral ischaemia remain largely unknown.

In conclusion, in our experimental methods, we have shown that desflurane affords a more effective protection than halothane against transient focal cerebral ischaemia in rats. Although clinical evidence is lacking, numerous experimental studies support the hypothesis that volatile anaesthetics are neuroprotective following cerebral ischaemia.

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