Sevoflurane pre- and post-conditioning protect the brain via the mitochondrial K\textsubscript{ATP} channel

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Background. This study aimed to evaluate whether exposure to sevoflurane at the onset of reperfusion provides protection similar to sevoflurane preconditioning and whether the effect depends on mitochondrial potassium ATP-dependent channel (mitoK\textsubscript{ATP}) in a rat model of focal cerebral ischaemia.

Methods. Adult Wistar male rats were subjected to focal cerebral ischaemia for 1 h followed by 24 h or 7 days of reperfusion. Preconditioning consisted of 15 min exposure to sevoflurane at 1 minimum alveolar concentration (2.6%) 72 h before ischaemia. Post-conditioning was performed by exposure to sevoflurane immediately at the onset of reperfusion or by a delayed exposure 5 min after the onset of reperfusion. The role of the mitoK\textsubscript{ATP} channel was assessed by i.p. injection of the selective blocker 5-hydroxydecanoate before each sevoflurane administration or by the mitoK\textsubscript{ATP} channel opener, diazoxide (DZX), given in place of sevoflurane. Cerebral infarct size, neurological deficit score, and motor coordination were evaluated 24 h and 7 days after reperfusion.

Results. Sevoflurane preconditioning and early post-conditioning reduced both cerebral infarct size and neurological defect score at 24 h of reperfusion whereas the sole sevoflurane post-conditioning improved motor coordination. At 7 days, only infarct volume remained lower in pre- and post-conditioned animals. Neuroprotection mediated by sevoflurane was lost when it was given 5 min after the onset of reperfusion and was abolished by inhibition of mitoK\textsubscript{ATP}. DZX alone mimicked sevoflurane-induced pre- and post-conditioning.

Conclusions. The pretreatment with sevoflurane or its early administration at reperfusion provides neuroprotection via mitoK\textsubscript{ATP} in a rat model of focal cerebral ischaemia.

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A brief period of ischaemia before cerebral ischaemia–reperfusion, known as ischaemic preconditioning (IPC), induces tolerance against ischaemic injury.\textsuperscript{1} Cerebral preconditioning can occur when an ischaemic signal precedes prolonged ischaemia by a few minutes (early preconditioning) or several days (delayed preconditioning). Volatile anaesthetics provide cerebral preconditioning in a manner similar to IPC.\textsuperscript{2}

Although preconditioning is an effective process for protection against cerebral damage, its clinical use is limited as ischaemic episodes are mostly unpredictable. However, the onset of reperfusion is more often predictable. Therefore, the concept of post-conditioning, through modulation of reperfusion rather than ischaemia, has been proposed, consisting in the introduction of ischaemic stimulus or volatile anaesthetics immediately at the onset of reperfusion to protect organs from ischaemia/reperfusion injuries. The protective effects of both ischaemic and anaesthetic post-conditioning have been demonstrated in the heart.\textsuperscript{3,4} Zhao and colleagues\textsuperscript{5} were the first to report that ischaemic post-conditioning can reduce cerebral ischaemic/reperfusion injury. Very recently, it was
shown that isoflurane administered after oxygen–glucose deprivation or brain ischaemia provided neuroprotection. Therefore, it remains to be confirmed whether the administration of volatile anaesthetics such as sevoflurane at the onset of reperfusion can provide similar neuroprotection than preconditioning.

Numerous studies found that volatile anaesthetic preconditioning confers cardiomyocyte protection via the mitochondrial K$_{ATP}$ channel (mitoK$_{ATP}$). Since volatile anaesthetic pre- and post-conditioning provide similar protection in the heart, they may share common pathways such as mitoK$_{ATP}$. In corticostriatal slices, K$_{ATP}$ channel inhibitors have been reported to block cell protection with isoflurane immediately after oxygen–glucose deprivation. Therefore, we hypothesize that sevoflurane post-conditioning-induced neuroprotection involves the mitoK$_{ATP}$ channel.

This study was designed to evaluate the neuroprotective effects induced by sevoflurane pre- and post-conditioning on cerebral infarct size in a model of rat cerebral focal ischaemia/reperfusion and studied the role of mitoK$_{ATP}$ by the modulation of these effects with either a selective mitoK$_{ATP}$ blocker, 5-hydroxydecanoate (5-HD), or a mitoK$_{ATP}$ channel opener, diazoxide (DZX).

Methods

All experiments were performed on male Wistar rats (Elevage Janvier, Le Genest Saint Isle, France) aged 10–12 weeks and weighing 280–320 g. Rats were housed in cages maintained at 20°C, with a 12 h day/night cycle and free access to water and food. All experiments were conducted in compliance with National regulations on the protection of animals used for experimental and other scientific purposes. Chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and Abbott for sevoflurane (Sevorane®).

Rats were anaesthetized with chloral hydrate (300 mg kg$^{-1}$, i.p.). Anaesthesia was ascertained by pedal withdrawal reflex. Central temperature was maintained at 37.0 (±0.5)°C using a heating lamp. Focal cerebral ischaemia was induced by reversible intraluminal middle cerebral artery occlusion as previously described.

Briefly, under an operating microscope, the right carotid bifurcation was exposed through a midline cervical incision. The external carotid was ligated distally. The pterygopalatine artery was ligated at its origin. An arteriotomy was performed in the common carotid artery which had been ligated more proximally, allowing the introduction of a 4/0 surgical nylon monofilament with its tip rounded by heat. The filament was gently advanced into the internal carotid artery 18–20 mm past the carotid artery bifurcation, thereby occluding the origin of the middle cerebral artery. One hour later, the filament was gently withdrawn to allow reperfusion. Animals were then placed in a cage to recover from anaesthesia at room temperature and were allowed food and drink.

The right common carotid artery was also cannulated under the proximal ligature to monitor arterial pressure and to draw arterial blood gases during ischaemia and reperfusion in additional rats from each group.

Twenty-four hours or 7 days after the 60 min ischaemia period, rats were killed by an overdose of pentobarbital. Brains were rapidly removed, frozen, and coronally sectioned into 50 μm thick slices on a cryostat as previously described. Sections were stained with cresyl violet and unstained area of the brain sections was considered as infarcted. Cortical, subcortical, and total hemispheric areas were measured after digitization, and then infarct volumes and hemisphere volumes were calculated by numerical integration of the respective areas as previously described.

A corrected total infarct volume was calculated to compensate for the effect of brain oedema using the following equation: corrected infarct volume=infarct volume−(right hemisphere volume−left hemisphere volume).

Neurological deficit scores (NDSs) were evaluated 24 h and 7 days after 60 min ischaemia based on an eight-point scale. The score was 0 for no apparent deficits; 1 for failure to extend left forepaw fully; 2 for decreased grip of the left forelimb; 3 for spontaneous movement in all directions, contralateral circling only if pulled by the tail; 4 for circling or walking to the left; 5 for walking only if stimulated; 6 for unresponsiveness to stimulation and with depressed level of consciousness; and 7 for death.

Motor co-ordination was evaluated 24 h before and 24 h and 7 day after 60 min ischaemia. The rats were placed on an accelerating rotarod. The speed of rotarod was increased from 4 to 40 rpm in 5 min. The time spent on the accelerating rotarod was recorded. All rats were trained for three continuous days before the formal tests.

Animals were randomly assigned to eight groups of at least five per group. Seven groups underwent interventions and one group served as a control (Fig. 1). In Group 1, rats were subjected to cerebral focal ischaemia as described above (ischaemia/reperfusion group, I/R). An additional control group was performed for neurological functional evaluation at 24 h and 7 days after 60 min ischaemia. Animals were killed at 7 days of reperfusion (Fig. 1A).

In Group 2, 72 h before ischaemia/reperfusion, animals were exposed to sevoflurane in a gas-tight anaesthesia chamber (230×125×120 mm plexiglass chamber). Rats spontaneously breathed for 15 min sevoflurane delivered to the gas admixture (oxygen) at a concentration of 2.6% via a calibrated vaporizer (Sevorane Vapor 15.3, Abbott) (sevoflurane late preconditioning group, S-PRE). A gas analyser (Capnomac Ultima, Datex Engström) was connected to the chamber to monitor and maintain inspired oxygen and sevoflurane concentrations constant. An additional sevoflurane-induced preconditioning was performed for neurological functional evaluation at 24 h and
Fig 1 Design protocol of study. (a) I/R, ischaemia/reperfusion group. (b) S-PRE, sevoflurane-induced preconditioning. Sevoflurane-induced immediate (c: S-POST) and delayed (d: delayed S-POST) post-conditioning. 5-HD + sevoflurane-induced preconditioning (e: S-PRE-HD) and post-conditioning (f: S-PO-HD). DZX-induced preconditioning (g: PRE-ZOX), post-conditioning (h: PO-ZOX). (i) Exposure to 95% oxygen: hyperoxia group.
7 days after 60 min ischemia. Animals were killed at 7 days of reperfusion (Fig. 1a).

In Group 3, rats were placed in the anaesthesia chamber and exposed for 15 min to sevoflurane 2.6% immediately at the onset of reperfusion (immediate sevoflurane-induced post-conditioning, S-POST) (Fig. 1c). Another group of rats were exposed to sevoflurane 2.6% 5 min after the onset of reperfusion (delayed sevoflurane-induced post-conditioning, delayed S-POST) (Fig. 1b). Rats were also exposed to oxygen alone (hyperoxia group, POX, Fig. 1i). An additional immediate sevoflurane-induced post-conditioning was performed for neurological functional evaluation at 24 h and 7 days after 60 min ischaemia. Animals were killed at 7 days of reperfusion.

The doses of 5-HD acid sodium salt (40 mg kg\(^{-1}\)) and DZX (10 mg kg\(^{-1}\)) were based on a previous study.\(^{14}\) The role of mitoK\(_{\text{ATP}}\) was investigated through i.p. injection of 5-HD, a selective mitoK\(_{\text{ATP}}\) blocker, 30 min before sevoflurane-induced preconditioning (5-HD+sevoflurane-induced preconditioning, S-PRE-HD) (Fig. 1i) and 30 min before immediate sevoflurane-induced post-conditioning (5-HD+sevoflurane-induced post-conditioning, S-PO-HD) (Fig. 1r).

To explore if opening the mitoK\(_{\text{ATP}}\) alone was able to mimic sevoflurane neuroprotection, DZX (i.p.), a mitoK\(_{\text{ATP}}\) channel opener, was administered in place of sevoflurane-induced preconditioning (DZX preconditioning, PRE-ZOX) (Fig. 1g) or in place of sevoflurane-induced post-conditioning (DZX post-conditioning, PO-ZOX) (Fig. 1h).

Because of DZX pharmacokinetics, i.p. injection was performed 30 min before reperfusion in the DZX post-conditioning group.

**Haemodynamics and arterial blood gas measurements**

Additional experiments were performed to evaluate the effects of ischaemia and reperfusion, sevoflurane, and pharmacological agents on mean arterial pressure (MAP, mm Hg), heart rate (HR, beats min\(^{-1}\)), arterial pH, arterial oxygen tension (\(P_{O2}\), mm Hg), and carbon dioxide tension (\(P_{CO2}\), mm Hg). In all rats, heart rate was monitored continuously. A peripheral arterial cannula was inserted for continuous monitoring of arterial pressure and for blood sampling. Arterial blood acid–base analysis was made using a Ciba Corning blood gas analyser (Halsted, Essex, UK), and the results were corrected for body temperature. Haemodynamic measurements were performed 15 and 35 min after the onset of ischaemia and 5 and 15 min after the onset of reperfusion. Arterial blood gas measurements were performed 15 min after the onset of ischaemia or reperfusion.

**Statistical analysis**

Data are expressed as median (inter-quartile range) or mean (SD). The Kruskal–Wallis test was used to test differences among the experimental groups. When differences among the groups were observed, a Mann–Whitney test was used to compare vs the I/R group. Significant differences were accepted when \(P<0.05\).

**Results**

Representative brain sections in the ischaemia/reperfusion and sevoflurane pre- and post-conditioning groups at 24 h and at 7 days after reperfusion are shown in Figure 2. Sevoflurane preconditioning reduced total infarct volume by 35% compared with the I/R group (\(P=0.019\)). Inhalation of sevoflurane during the first 15 min of reperfusion, immediate post-conditioning, decreased cerebral infarction by 45% (\(P=0.001\)) whereas hyperoxia alone did not provide any protection (Fig. 3a). NDSs in the S-PRE and in S-POST groups at 24 h after reperfusion were lower than those of I/R (Fig. 4a) whereas motor coordination was only improved in S-POST (Fig. 4n). The protective effect was lost when sevo- flurane administration was delayed by 5 min after the onset of reperfusion, in delayed post-conditioning (Fig. 3a). Reduction in infarct volume involved both the cortex and not the striatum.

**MitoK\(_{\text{ATP}}\) in sevoflurane-induced neuroprotection**

Cerebral infarct size in S-PRE and S-POST pretreated by 5-HD was not different compared with the I/R group.
Moreover, administration of 5-HD completely blocked the protection observed in S-POST. In S-PRE, 5-HD partially reversed the protection as the difference between the two groups did not reach significance. DZX given in place of sevoflurane mimicked sevoflurane pre- and post-conditioning. DZX administered 72 h before ischaemia/reperfusion reduced infarct volume by 24.6% (Fig. 5B), whereas DZX administration 30 min before reperfusion decreased infarct volume by 52%.

Pharmacological preconditioning induced by DZX concerned mainly the cortex without any effect on the striatum or oedema. In the PO-ZOX group, protection was located in the cortex but not in the striatum and led to a significant reduction in oedema of 57% ($P=0.001$).

**Arterial blood gas and haemodynamic data**

There were no significant differences in arterial pH and $P_{CO_2}$ during ischaemia and during reperfusion between the groups (data not shown). Immediate or delayed sevoflurane post-conditioning led to increased $P_{O_2}$ during reperfusion ($P<0.05$) [S-POST 70 (5) vs 188 (55) mm Hg and delayed S-POST 73 (9) vs 503 (10) mm Hg] (Table 1). No hypotension was observed during administration of sevoflurane or pharmacological agents (Table 2).

**Discussion**

This work demonstrates that sevoflurane delayed preconditioning and early post-conditioning reduced brain infarct volume and NDS 24 h after reperfusion in a rat model of transient focal cerebral ischaemia. Motor coordination was only improved for sevoflurane post-conditioning. The reduction of cerebral infarct size was prolonged until 7 days after reperfusion. In contrast, no protection was observed when sevoflurane was administered 5 min after the onset of reperfusion. Neuroprotection involves mitoKATP as the blockage of the channel abolished the protection and mitoKATP agonist given before ischaemia or at the onset of reperfusion mimicked it.

Neuroprotective effects of pretreatment with volatile anaesthetics including sevoflurane have already been observed in both in vivo models of global and focal cerebral ischaemia and in vitro models of neuronal cell culture. Sevoflurane was administered 72 h before a cerebral ischaemia corresponding to a delayed window of protection or late preconditioning. This differs from early preconditioning in which the application of triggering stimulus is carried out within minutes before ischaemia. The sevoflurane pretreatment protocol used was based on the IPC model currently used by our group. Cerebral infarct volumes were demonstrated to be reduced when IPC was performed 24 or 72 h before prolonged ischaemia. A more robust protection was observed during the 72 h time-window, whereas no preconditioned effect occurred 30 min before ischaemia. The sevoflurane dose chosen, 2.6% or 1 minimum alveolar concentration, was that which conferred the maximal neuroprotective effects as previously determined in IPC experiments.
The major novel finding of our study was that sevoflurane provided neuroprotection when it was inhaled at the onset of reperfusion. To our knowledge, this is the first time that sevoflurane given at the onset of reperfusion was reported to provide a reduction of focal cerebral ischaemic/reperfusion injury. Lee and colleagues \cite{6} reported post-conditioning with isoflurane via a tracheal tube for 60 min after 90 min middle cerebral arterial occlusion in rats. The limitation of this work is that rats were anaesthetized with isoflurane during the procedure of middle cerebral arterial occlusion in rats. The limitation of this work is that rats were anaesthetized with isoflurane during the procedure of middle cerebral arterial occlusion in rats. Numerous studies have demonstrated that sevoflurane pre- and post-conditioning provided similar significant reductions in cardiac infarct size when compared with a control group. \cite{4, 19} The sevoflurane post-conditioning group showed similar reductions in cerebral ischaemic/reperfusion damage when compared with the sevoflurane preconditioning group.

Reduced injured brain tissue was associated with an improvement of neurological deficit assessed by NDS 24 h after reperfusion in the sevoflurane pre- and post-conditioning groups. This is in agreement with the fact that neurological grades have been shown to correlate with the volume of hemispheric infarction. \cite{12} Therefore, the decrease of cerebral infarct volumes in the I/R group explains at least in part the lack of difference on motor deficit between the groups 7 days after reperfusion, whereas the ischaemic damage remained significantly lower with sevoflurane pre- and post-conditioning. In addition, cerebral oedema plays an important role on the severity of motor deficit as a relationship has been demonstrated between brain oedema and motor deficits in focal ischaemia. \cite{20} The best motor performance on the rotarod task at 24 h after reperfusion with sevoflurane post-conditioning could be related to the significant 40% reduction in cerebral oedema in this group. Whether

**Fig 4** Evaluation of neurological outcome. (A) NDS at 24 h (H24) and on day 7 (D7); values are median and inter-quartile range. \( *P<0.05 \) and \( **P<0.01 \) vs the I/R group. (B) Rotarod performance: duration (s) spent on rotarod at 24 h on day 7. Values are median and inter-quartile range. \( *P<0.05 \) vs the I/R group. \( n=8 \) for each group.
Implication of diazoxide during anaesthetic pre- and post-conditioning

Effect of 5HD on anaesthetic pre and post-conditioning

Fig 5 Role of mito-K<sub>ATP</sub> channel in anaesthetic pre- or post-conditioning with sevoflurane is tested using (A) channel antagonist 5-HD or (B) selective channel agonist DZX. Total infarct volume is corrected for brain oedema. Values are median and inter-quartile range. *P<0.05 vs I/R, **P<0.01 vs I/R.
A previous study using an antioxidant cocktail given at the onset of reperfusion reported significant protection. However, we did not assess reactive oxygen species generation in our study, so the potential role of sevoflurane in attenuation of oxidative burst is not known.

The implication of mitoK<sub>ATP</sub> channel was mainly reported in IPC in models of occlusion of the middle cerebral artery, and neuronal culture. In rat hippocampal slices, the preconditioning induced by sevoflurane given 30 min before hypoxia was abolished by 5-HD, a mitoK<sub>ATP</sub> inhibitor. Similarly, we observed that mitoK<sub>ATP</sub> channel inhibition blocked sevoflurane preconditioning, whereas mitoK<sub>ATP</sub> channel opening mimicked late preconditioning induced by sevoflurane. Likewise, sevoflurane post-conditioning was also blocked by 5-HD given at the end of ischaemia and that administration of DZX instead of sevoflurane conferred neuroprotection similar to sevoflurane. Recently, in brain slices, glibenclamide, a general K<sub>ATP</sub> inhibitor, and 5-HD attenuated the cell protection induced by isoflurane post-conditioning. Collectively, these results suggest that volatile anaesthetic-induced post-conditioning may be mediated by the mitoK<sub>ATP</sub> channel.

The mechanism by which the mitoK<sub>ATP</sub> channel is involved in anaesthetic pre- and post-conditioning is still not understood. Sevoflurane pre- and post-conditioning result in preservation of mitochondrial function and maintenance of ATP stores. It has been proposed that opening of the mitoK<sub>ATP</sub> channel is associated with an uptake of potassium in the mitochondrial matrix which could maintain mitochondrial matrix volume by attenuating Ca<sup>2+</sup> loading. This reduction of mitochondrial Ca<sup>2+</sup> load would prevent, during reperfusion, the opening of the mitochondrial permeability transition pore known to inhibit oxidative phosphorylation and facilitate the release of proapoptotic proteins.

Involvement of apoptotic mechanisms in protection induced by sevoflurane pre- and post-conditioning is consistent with the fact that infarct size remained lower than I/R 7 days after reperfusion. Although the mitoK<sub>ATP</sub> channel appears to be involved in sevoflurane pre- and post-conditioning, future progress in revealing the mechanism of anaesthetic neuroprotection will require a clearer understanding of the chain of events and molecular pathways that prevent apoptosis.

### Table 1
Gazometric data for experimental groups during postconditioning; results are expressed as mean (SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>pH</th>
<th>PO&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</th>
<th>PCO&lt;sub&gt;2&lt;/sub&gt; (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/R</td>
<td>7.35 (0.02)</td>
<td>77 (4)</td>
<td>47 (2)</td>
</tr>
<tr>
<td>S-POST</td>
<td>7.36 (0.01)</td>
<td>72 (2)</td>
<td>46 (1)</td>
</tr>
<tr>
<td>Delayed S-POST</td>
<td>7.34 (0.01)</td>
<td>70 (5)</td>
<td>48 (2)</td>
</tr>
<tr>
<td>S-POST+5-HD</td>
<td>7.32 (0.02)</td>
<td>188 (55)</td>
<td>53 (1)</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>7.34 (0.01)</td>
<td>73 (9)</td>
<td>51 (2)</td>
</tr>
<tr>
<td></td>
<td>7.30 (0.03)</td>
<td>303 (10)</td>
<td>63 (6)</td>
</tr>
<tr>
<td></td>
<td>7.31 (0.01)</td>
<td>64 (2)</td>
<td>56 (2)</td>
</tr>
<tr>
<td></td>
<td>7.34 (0.04)</td>
<td>81 (4)</td>
<td>52 (6)</td>
</tr>
<tr>
<td></td>
<td>7.32 (0.02)</td>
<td>60 (5)</td>
<td>56 (3)</td>
</tr>
<tr>
<td></td>
<td>7.34 (0.02)</td>
<td>71 (6)</td>
<td>53 (3)</td>
</tr>
</tbody>
</table>

### Table 2
Haemodynamic data (mean arterial pressure, MAP and heart rate, HR) at different times for experimental groups during postconditioning; results are expressed as mean (SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>t+15 min of ischaemia</th>
<th>t+35 min of ischaemia</th>
<th>t+5 min of reperfusion</th>
<th>t+15 min of reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/R</td>
<td>89 (1)</td>
<td>89 (2)</td>
<td>88 (5)</td>
<td>86 (5)</td>
</tr>
<tr>
<td>HR (beats min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>338 (9)</td>
<td>338 (8)</td>
<td>334 (12)</td>
<td>334 (12)</td>
</tr>
<tr>
<td>S-POST</td>
<td>88 (4)</td>
<td>87 (4)</td>
<td>80 (3)</td>
<td>81 (4)</td>
</tr>
<tr>
<td>HR (beats min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>318 (9)</td>
<td>326 (18)</td>
<td>262 (18)</td>
<td>284 (24)</td>
</tr>
<tr>
<td>Delayed S-POST</td>
<td>89 (9)</td>
<td>82 (7)</td>
<td>87 (10)</td>
<td>86 (4)</td>
</tr>
<tr>
<td>HR (beats min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>334 (14)</td>
<td>312 (28)</td>
<td>312 (18)</td>
<td>276 (18)</td>
</tr>
<tr>
<td>S-POST+5-HD</td>
<td>87 (4)</td>
<td>90 (5)</td>
<td>82 (1)</td>
<td>85 (4)</td>
</tr>
<tr>
<td>HR (beats min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>309 (12)</td>
<td>314 (10)</td>
<td>232 (22)</td>
<td>240 (27)</td>
</tr>
<tr>
<td>Diazoxide</td>
<td>86 (6)</td>
<td>80 (9)</td>
<td>82 (9)</td>
<td>82 (9)</td>
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<tr>
<td>HR (beats min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>296 (11)</td>
<td>298 (12)</td>
<td>290 (26)</td>
<td>304 (31)</td>
</tr>
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</table>
Our study has several limitations. First, sevoflurane was administered in 95% inspired fraction of oxygen leading to hyperoxia, shown also to be neuroprotective. In our study, implication of hyperoxia in neuroprotection is unlikely, since hyperoxia alone for 15 min at the onset of reperfusion did not confer any protection. Secondly, spontaneously breathing anaesthetized rats provided a moderate respiratory acidosis due to alveolar hypoventilation. In acid conditions (pH=6.6), 5-HD could also block the sarcolemmal potassic ATP-dependent channel implicated recently in isoflurane-induced preconditioning in rat cardiomyocytes. However, the low pH decrease reaching a maximum value at 7.3 was probably not sufficient for a non-specific action of 5-HD. Moreover, administration of DZX, an agonist of mitoKATP, mimicked neuroprotection, suggesting the direct implication of the channel.

Overall, this study demonstrates neuroprotective properties of sevoflurane in an in vivo model. Exposure to sevoflurane 72 h before ischaemia or immediately at reperfusion reduces the size of cerebral infarct and improves neurological function. This neuroprotection appears to be mediated by the mitoKATP channel which could interfere with pathways leading to neuronal death. Although the relationship between sevoflurane and mitoKATP channel needs to be clarified through future studies, a focus should be kept on mitochondria which seem to play a crucial role in neuroprotection.

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References
2 Kapinya Kj, Lowl D, Futterer C, et al. Tolerance against ischemic neuronal injury can be induced by volatile anesthetics and is inducible NO synthase dependent. Stroke 2002; 33: 1889–98
5 Zhao H, Sapolsky RM, Steinberg GK. Interrupting reperfusion as a stroke therapy: ischemic postconditioning reduces infarct size after focal ischemia in rats. J Cereb Blood Flow Metab 2006; 26: 1114–21
7 Zuegg M, Lucchinietti E, Spahn DR, Pasch T, Scharp MC. Volatile anesthetics mimic cardiac preconditioning by priming the activation of mitochondrial K(ATP) channels via multiple signaling pathways. Anesthesiology 2002; 97: 4–14
18 Puisieux F, Deplanque D, Bulkaen H, et al. Brain ischemic preconditioning is abolished by antioxidant drugs but does not up-regulate superoxide dismutase and glutathione peroxidase. Brain Res 2004; 1027: 30–7


