Sevoflurane preconditioning-induced neuroprotection is associated with Akt activation via carboxy-terminal modulator protein inhibition

Y. Chen†, H. Nie†, L. Tian, L. Tong, J. Deng, Y. Zhang, H. Dong* and L. Xiong*

1 Department of Anaesthesiology and 2 Department of Orthopaedics, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710032, China

* Corresponding author. E-mail: hldong6@hotmail.com (H.D.); mzxlz@126.com (L.X.)

Editor’s key points

- The mechanisms by which sevoflurane preconditioning protects against cerebral ischaemia are unclear.
- In a rat model of focal cerebral ischaemia, sevoflurane preconditioning reduced infarct size and neurological dysfunction.
- The protective effect involved preservation of Akt signalling by down-regulation of an endogenous inhibitor.
- Identification of this inhibitor reveals a novel target for neuroprotective drugs.

More than 2 million major surgical operations are performed every year worldwide, with increasing frequency of reported perioperative cerebral ischaemia.1 Preconditioning with anaesthetics against ischaemic neuronal injury is effective in vitro and in vivo.2–6 With few clinical side-effects, the commonly used volatile anaesthetic sevoflurane has received increasing attention in this domain.5–8 Neuroprotective strategies such as ischaemic preconditioning and other neuroprotective strategies promote neuronal survival by activating the protein kinase Akt.9–13 Upon activation, Akt phosphorylates/inactivates downstream targets such as GSK3β implicated in both caspase-dependent and -independent mechanisms of cell death.12–15 Carboxy-terminal modulator protein (CTMP), a novel Akt binding protein,16 binds to the carboxyl-terminal regulatory domain of Akt at the plasma membrane, and thereby negatively regulates the activity of Akt by inhibiting phosphorylation on serine 473 and threonine 308.16 During global cerebral ischaemic injury, CTMP is highly up-regulated and reduces Akt signalling,17 thus contributing to ischaemic neuronal death. The present study tested the hypotheses that sevoflurane preconditioning inhibits expression of CTMP triggered by ischaemia, thereby preserving the activity of Akt and decreasing infarct volume.

Methods

All experiments were conducted with the approval of the Ethics Committee for Animal Experimentation of the Fourth Military Medical University (Xi’an, China) and in accordance with the...
National Institutes of Health Guide for the Care and Use of Laboratory Animals and ARRIVE guidelines. Adult male Sprague–Dawley rats (200–250 g) were purchased from the Laboratory Animal Centre of our university.

**Experimental protocols**

**Experiment I**

To investigate the neuroprotective effect of sevoflurane preconditioning against focal cerebral ischaemia, rats were divided into four groups: sham operation (Sham), cerebral ischaemia/reperfusion (I/R), sevoflurane preconditioning (PC), and preconditioning followed by cerebral ischaemia/reperfusion (PC + I/R) groups (n = 7 each). Preconditioned animals were exposed to 2.7% sevoflurane for 45 min at 1 h before ischaemia.\(^6\) Focal cerebral ischaemia was induced by middle cerebral artery occlusion (MCAO). Neurological deficits and infarct volumes were evaluated at 1 and 7 days after reperfusion (Fig. 1A).

To determine the effect of sevoflurane preconditioning on the phosphorylation of Akt and its downstream target GSK3\(^\beta\), and also changes in CTMP levels after ischaemia, rats in the PC group were killed 24 h after preconditioning. Rats in the I/R and PC + I/R groups were killed at 1, 3, 12, and 24 h after reperfusion (n = 5 each). Akt activity was determined 3 h after reperfusion (n = 4).

**Experiment II**

To investigate the role of phosphoinositide 3-kinase (PI3K)/Akt pathways in the induction of ischaemic tolerance by sevoflurane preconditioning, we randomly assigned 80 rats to eight groups: I/R, PC + I/R, sevoflurane preconditioning with PI3K inhibitor wortmannin (WT + PC + I/R), sevoflurane preconditioning with PI3 inhibitor LY294002 (LY + PC + I/R), ischaemia with wortmannin (WT + I/R), ischaemia with LY294002 (LY + I/R), and two vehicle (VE-WT + PC + I/R and VE-LY + PC + I/R) groups. Wortmannin and LY294002 (Sigma-Aldrich, St Louis, MO, USA) were administered 10 min before preconditioning (Fig. 1A). Wortmannin (0.6 mg kg\(^{-1}\) in saline) or its vehicle was administered via tail vein;\(^18\) LY294002 (25 \(\mu\) g in 5 \(\mu\) l 1% DMSO) or its vehicle was administered intracerebroventricularly (coordinates: 0.8 mm anteroposterior, 1.5 mm lateral to bregma, and 3.5 mm in deep).\(^19\)

**Experiment III**

To examine the effect of CTMP in sevoflurane preconditioning and I/R, we used lentiviral transduction (LV) to augment expression of CTMP before preconditioning. Rats received an intracerebroventricular (5 \(\mu\) l) injection of either LV-CTMP or LV-C (scrambled control vector) 3 days before preconditioning. After 24 h of reperfusion, animals were evaluated for neurological scores and then killed to determine infarct volume (n = 7) (Fig. 1C). Validation for lentiviral transduction was carried out at 72 h after intracerebroventricular injection by determining the expression of CTMP by immunoblotting and Akt activity with a kinase assay kit (Cell Signalling Technology, Beverly, MA, USA) in rats without MCAO (n = 3).

**Sevoflurane preconditioning**

Sevoflurane preconditioning was performed as previously described, with some modifications.\(^6\) Briefly, preconditioned animals were exposed to 2.7% sevoflurane (Baxter Healthcare Corporation, Chicago, IL, USA) in 97% oxygen for 45 min in a chamber. Concentrations of oxygen, carbon dioxide, and sevoflurane were continuously monitored (MP-60, Phillips Medical Systems, Best, The Netherlands). Control group rats were treated for the same duration with 97% oxygen only.

**MCAO model**

MCAO was performed as previously described.\(^6\)\(^,\)\(^20\) Briefly, after rats were anaesthetized with 3.0% sevoflurane, transient focal cerebral ischaemia was induced by occlusion of the right middle cerebral artery using an intraluminal 0.38 mm diameter

---

**Fig 1.** (a) Graphical representation of the protocol for experiment I shows that neurological deficits and infarct volumes were evaluated at 1 and 7 days after reperfusion to investigate the neuroprotective effect of sevoflurane preconditioning against focal cerebral ischaemia. Expression of pSer473-Akt, pSer9 GSK-3\(^\beta\), and CTMP were analysed at different time points after reperfusion. (a) Graphical representation of the protocol for experiment II shows that PI3K inhibitor wortmannin and LY294002 were used before sevoflurane preconditioning to investigate the role of PI3K/Akt in the induction of ischaemic tolerance. (c) Graphical representation of the protocol for experiment III shows that CTMP and scramble lentivirus transduction were used 3 days before preconditioning to investigate the effect of over-expression of CTMP on sevoflurane-induced neuroprotection. Sevo, sevoflurane preconditioning; LV-CTMP, lentiviral vector Ubi-MCS-CTMP; LV-C, lentiviral vector-scramble.
nylon monofilament suture (Beijing Sunbio Biotech Co. Ltd, Beijing, China). After 60 min of occlusion, the monofilament was removed to allow reperfusion. The left femoral artery was cannulated to monitor arterial pressure and to draw blood samples for gas determination (Rapidlab 1260, Bayer HealthCare, Uxbridge, UK) at the beginning of ischaemia and at the onset of reperfusion. Regional cerebral blood flow (rCBF) was measured by a laser-Doppler computerized main unit (PeriFlux 5000, Perimed AB, Stockholm, Sweden) with a disposable microtip fibroprobe placed on the skull (6 mm lateral and 1 mm posterior to bregma). If rCBF sharply decreased by more than 70% of the baseline (pre-ischaemia) after MCAO and was rapidly restored during reperfusion (in 20 min), the model was considered technically successful. During the surgical procedure, systemic temperature was monitored with a rectal probe and maintained at 37.0 (0.5 °C).

**Neurobehavioural evaluation and infarct assessment**

Neurological dysfunction was evaluated according to an 18-point scoring system by an investigator who was blinded to the animal grouping. To evaluate infarct volume, animals were killed for 2,3,5-triphenyltertrazolium chloride (TTC, Sigma-Aldrich) staining as described. Infarct volume was calculated using Swanson’s method: (contralateral hemisphere volume–ipsilateral hemisphere volume)/contralateral hemisphere volume × 100%.  

**Immunoblotting**

Immuno blotting was performed with a routine protocol. Briefly, brain tissue from the ischaemic penumbra as defined by the Ashwal and colleagues method was quickly isolated and snap-frozen. Protein was extracted using RIPA lysis buffer (Beyotime, Nantong, China). Polyacrylamide–SDS gels with an equal amount of protein (40 μg) loaded in each lane were electrophoresed, and the proteins transferred to a PVDF membrane. After incubation with 5% bovine serum albumin in Tris-buffered saline solution with 0.05% Tween-20 for 1 h at 4 °C, the membrane was probed with primary antibodies (Cell Signalling Technology): rabbit anti-pAkt Ser473 or Thr308 antibodies (1:1000 for both), rabbit anti-Akt antibody (1:2000), rabbit anti-P-GSK3β Ser9 antibody (1:1000), rabbit anti-GSK3β antibody (1:2000), or rabbit anti-CTMP antibody (1:500). After rinsing, membranes were probed with HRP-conjugated anti-rabbit antibody for 2 h at room temperature. Immunoreactive bands were detected with enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, UK) and quantified with Bio-Rad Quantity One software (Bio-Rad, Hercules, CA, USA).

**Akt activity assays**

In vitro Akt kinase assays were performed with an Akt kinase assay kit as described. In brief, brain tissue from the ischaemic penumbra (100–120 mg) was homogenized in seven volumes of cell lysis buffer. After centrifugation at 14 000g for 10 min at 4 °C, the supernatant (whole-cell extract) was taken for the kinase assay. For immunoprecipitation with immobilized Akt primary antibody, cell lysate (200 μl, 150 μg of protein) and 20 μl of immobilized antibody bead slurry were incubated with gentle rocking overnight at 4 °C. To confirm that each group had the same amount of extraction, the upper solution (solution I) was saved for GAPDH detection by immunoblotting after centrifugation at 14 000g for 30 s at 4 °C. The pellet was washed twice with cell lysis buffer and kinase buffer on ice, and then incubated with 50 μl of kinase buffer supplemented with 1 μl of 10 mM ATP and 1 μg of GSK3 fusion protein for 30 min at 30 °C. To terminate the reaction, 25 μl 3 × SDS sample buffer was added. After centrifugation for 30 s at 14 000g, the supernatant was taken for immunoblotting detection of phosphorylated GSK3 fusion protein.

**Lentivirus-based over-expression of CTMP**

Transfection of the CTMP over-expression lentiviral vector Ubi-MCS-CTMP was performed as described. The complete rat cDNA sequence of CTMP was generated by PCR and inserted into the Ubi-MCS-3FLAG Vector (GeneChem, Shanghai, China), linearized with AgeI and NheI. Expression of the resulting 28 kDa protein was confirmed by immunoblotting analysis. The lentiviral vector was produced by co-transfection into HEK 293T-cells with a helper construct. Titers of 2 × 109 IU ml⁻¹ were routinely achieved.

**Statistical analysis**

All data, except neurological scores, are expressed as mean (so). Neurological scores were expressed by median (range) and analysed using the non-parametric Mann–Whitney test in experiment I or the Kruskal–Wallis test followed by Dunn’s post hoc test in other experiments. Infarct volumes in experiment I were analysed using a two-tailed t-test. Other experiments were analysed using repeated-measures one-way analysis of variance (ANOVA) with Tukey’s post hoc test. Arterial pressures and blood gases were analysed by a two-way ANOVA analysis, followed by the Bonferroni test for multiple comparisons. Data analyses were performed with the GraphPad Prism (Version 6, GraphPad Software Inc., San Diego, CA, USA). Statistical significance was defined as P < 0.05.

**Results**

Physiological variables were normal and equivalent in all study groups (Supplementary Tables S1–S3). Rats with inadequate rCBF decrease in the MCAO model or that died before the observation time point were excluded.

**Sevoflurane preconditioning induced neuroprotection**

Sevoflurane preconditioning significantly ameliorated neurological dysfunction induced by MCAO at 24 h after ischaemia [P < 0.05, 13.0 (9, 15) vs 9.0 (6, 11), Fig. 2A]. Infarct volume of rats in the PC+I/R group was smaller than that in the I/R group at both 24 h and 7 days after ischaemia [P < 0.01, 23 (6)% vs 36 (8)% at 24 h; P < 0.01, 27 (6)% vs 41 (9)% at 7 days, Fig. 2B and C]. Sevoflurane preconditioning reduced infarct volume nearly 13% compared with animals without preconditioning.
Sevoflurane preconditioning prevented the decrease in Akt activity and GSK3β phosphorylation and the increase in CTMP induced by ischaemia

Ischaemia induced a transient increase in the phosphorylation of Akt at Ser473 (Ser473-Akt), which peaked at 3 h after reperfusion \([P<0.01\text{ vs }\text{sham}, 250 (40)\%\text{ at }1\text{ h}, \text{ and }330 (58)\%\text{ at }3\text{ h}\) after reperfusion, Fig. 3a) and declined to basal levels 24 h after reperfusion \([P>0.05\text{ vs }\text{sham}, 110 (44)\%\text{ at }24\text{ h after reperfusion, Fig. 3a}]\). Phosphorylation of GSK3β (pSer9-GSK3β), a downstream target of Akt, was higher in the PC + I/R group than in the I/R group at 1 and 3 h after reperfusion \([P<0.05, 190 (33)\%\text{ vs }1120 (30)\%\text{ at }1\text{ h}; P<0.01, 150 (24)\%\text{ vs }60 (44)\%\text{ at }3\text{ h, Fig. 4a}]\). Phosphorylation of Akt and GSK3β were increased at 3 h after sevoflurane treatment compared with the sham group \([P<0.05, 190 (16)\%\text{ for Ser473-Akt}; P<0.05, 160 (21)\%\text{ for Ser9-GSK3β}, Fig. 4a and a]\).

Akt kinase activity assay showed that sevoflurane preconditioning increased Akt activity 3 h after reperfusion compared with the I/R group \([P<0.01, 200 (47)\%\text{ vs }81 (33)\%, \text{ Fig. 3b})\], parallel to increased pSer9-GSK3β in vivo. No significant differences were found between the Sham and I/R groups. When rats were subjected to ischaemia, expression of CTMP in penumbra tissues began to increase at 3 h after reperfusion compared with the Sham group \([P<0.01, 210 (52)\%\text{ at }3\text{ h, Fig. 3c})\] and remained higher than that of the sham group at 24 h after ischaemia \([P<0.01, 250 (42)\%\text{ at }12\text{ h and }230 (35)\%\text{ at }24\text{ h, Fig. 3c}]\). Sevoflurane preconditioning suppressed the increase in CTMP expression induced by I/R at 3, 12, and 24 h after reperfusion \([P<0.01, 100 (55)\%\text{ vs }210 (52)\%\text{ at }3\text{ h}; P<0.01, 140 (46)\%\text{ vs }250 (42)\%\text{ at }12\text{ h}; P<0.05, 140 (34)\%\text{ vs }230 (35)\%\text{ at }24\text{ h, Fig. 3c}]\). Sevoflurane alone did not inhibit CTMP expression significantly compared with the Sham group \([P>0.05, \text{ Fig. 3c}]\).

Over-expression of CTMP by lentiviral transduction prevented the neuroprotective effect of sevoflurane

Injection of LV-CTMP led to \(\sim 2.6\)-fold increase in CTMP protein expression in the targeted brain regions in rats without MCAO \([P<0.01\text{ vs Sham, Fig. 5a})\text{ at }72\text{ h after intracerebroventricular injection as defined by immunoblot analysis. Akt activity decreased nearly by half at the same time point }P<0.01\text{ vs Sham, Fig. 5a}]\). Although it did not further aggravate neurological dysfunction or infarct sizes, CTMP over-expression markedly attenuated the beneficial effects of sevoflurane preconditioning at 24 h after reperfusion as demonstrated by infarct volume \([P<0.05, 38 (10)\%\text{ in LV-CTMP + PC + I/R vs }24 (8)\%\text{ in PC + I/R}, \text{ Fig. 6a and c}]\). No significant differences were found in the neurological scores (Fig. 6a).

Activation of PI3K/Akt is involved in the neuroprotective effect of sevoflurane

Pretreatment with wortmannin or LY294002 totally abolished the neuroprotective effects of sevoflurane preconditioning. Compared with the PC + I/R group, pretreatment with wortmannin increased infarct sizes \([P<0.01 41 (13)\%\text{ vs }23 (8)\%, \text{ Fig. 4b and c})\] and reached the level of animals without sevoflurane preconditioning \([41 (13)\%\text{ for the I/R group}]\). Similar results were found with LY294002 as 41 (13)\% infarct size in the LY + PC + I/R group \((P<0.01)\). However, no significant differences were found in neurobehavioural scores (Fig. 4a).

Discussion

We show that sevoflurane preconditioning inhibits increased expression of CTMP elicited by brain ischaemia and thereby preserves the kinase activity of Akt to phosphorylate/inactive its downstream pro-apoptotic target GSK3β. Thus, inhibition of the endogenous Akt inhibitor CTMP is crucial for the neuroprotective effect of sevoflurane preconditioning.

Multiple molecules such as free radicals, ATP-regulated potassium channels \((K_{ATP})\), adenosine A1 receptors, mitogen-activated protein kinase, hypoxia-inducible factor-1α, and antioxidant enzymes have been implicated in the neuroprotection provided by anaesthetic-induced preconditioning (APC). These are important neuroprotective mechanisms, but we are still not clear about whether or not the classical
Fig 3 Effect of sevoflurane preconditioning on the expression of pSer473-Akt, pSer9 GSK-3β, and CTMP at 1, 3, 12, and 24 h after reperfusion ($n=5$, except I/R and PC+I/R at 24 h, $n=4$). Sevoflurane preconditioning suppressed hyperphosphorylation of Akt induced by focal cerebral ischaemia (A) and promoted phosphorylation of an Akt target GSK3β at Ser9 (B) at 1 and 3 h after reperfusion. Elevation of CTMP expression induced by focal cerebral ischaemia in penumbra tissues was inhibited by sevoflurane preconditioning at 3 and 12 h after reperfusion (C). Sevoflurane preconditioning increased Akt activity of penumbra tissues 3 h after reperfusion, whereas ischaemia did not (D). Significance of experimental vs sham animals is denoted by asterisk; PC+I/R vs I/R is denoted by hash. *$P<0.05$, **$P<0.01$. 

Role of CTMP in sevoflurane neuroprotection
pro-survival (anti-apoptotic) pathway PI3K/Akt/GSK3β is involved. We found that activation of PI3K/Akt is essential to induction of ischaemic tolerance by sevoflurane preconditioning in the MCAO model. Cerebral ischaemia induced a transient robust increase in phosphorylation of Akt at Ser473. Surprisingly, despite the increase in phosphorylation, the activity of Akt did not change. Sevoflurane preconditioning inhibited the Akt hyperphosphorylation evoked by ischaemia and increased Akt activity, which in turn phosphorylated/inactivated its downstream target GSK3β, thereby preserving more neurones. Activation of the PI3K/Akt pathway is important for sevoflurane preconditioning-induced neuroprotection.

A role for mitoKATP in the neuroprotective effect of sevoflurane preconditioning has been reported. It has been proposed that opening of the mitoKATP channel is associated with a reduction in mitochondrial Ca²⁺ load to prevent the opening of the mitochondrial permeability transition pore. A study of sevoflurane-induced cardioprotection showed that mitoKATP activation is mediated by activation of PI3K/Akt, indicating crosstalk between Akt and mitoKATP. In addition, PI3K/Akt is involved in regulation of expression of hypoxia-inducible factor-1α under both normoxic and hypoxic conditions. Pro-survival function of restored PI3K/Akt activity by APC might be associated with these mechanisms, but further studies are required.

Akt is phosphorylated on threonine 30, by PDK1, a PI3K-dependent protein kinase, and on serine 473 by a putative serine 473 kinase. Akt phosphorylation is negatively regulated...
Role of CTMP in sevoflurane neuroprotection

by two endogenous inhibitors: CTMP and PTEN (phosphatase and tensin homologue deleted on chromosome 10). PTEN inhibits Akt phosphorylation and activation by dephosphorylating phosphatidylinositol-3,4,5-triphosphate. In our study, phosphorylation of PTEN showed no obvious changes in all groups (data not shown), whereas increased expression of CTMP in the I/R group mirrored the loss of Akt kinase activity during reperfusion. Our findings are consistent with a model whereby ischaemia triggers hyperphosphorylation of Akt, which in turn promotes induction of CTMP, acting as a brake to inhibit Akt activity. We found that sevoflurane preconditioning suppressed the increase in CTMP expression after reperfusion, thus restoring the activity of Akt and promoting phosphorylation/inactivation of its downstream target, GSK3β. To get further solid evidence, we used lentiviral vectors encoding CTMP to observe the influence of CTMP over-expression on neuroprotection by sevoflurane preconditioning in vivo. Injection of LV-CTMP in normal animals without MCAO increased CTMP expression injection, mimicking increase in CTMP in rats with cerebral ischaemia. In ischaemic animals treated with sevoflurane PC, administration of LV-CTMP reversed the beneficial effects of sevoflurane preconditioning as assessed by infarct volume. In addition, sevoflurane alone decreased expression of CTMP at 3 h after treatment, and increased pAkt and pGSK3β. The underlying mechanism of regulation by sevoflurane of CTMP is currently unknown.

It is necessary to explain the absence of effects on post-reperfusion behaviours and infarct volumes in the non-preconditioned animals with PI3K/Akt or CTMP manipulation. Cerebral ischaemia induced both an up-regulation of ser473 Akt phosphorylation and increase in CTMP expression. However, activity of Akt did not show a detectable change after cerebral ischaemia. As we postulated, increased CTMP expression inhibits Akt activity after cerebral ischaemia, which resulted in inhibition of the pro-survival function of Akt. This is supported by lack of change in GSK3β phosphorylation. As shown in previous reports, the activity of Akt kinase is reflected by the phosphorylation of GSK3β but not its phosphorylation level. Sevoflurane preconditioning reduced CTMP expression and restored Akt activity after cerebral ischaemia, resulting in amelioration of ischaemic injury. Inhibition of PI3K by wortmannin or LY294002 or over-expression CTMP by lentiviral transduction reversed the neuroprotective effect of sevoflurane preconditioning. However, inhibition of PI3K or overexpression of CTMP alone in ischaemic animals, without preconditioning, did not further worsen ischaemia injury. One tentative explanation is that this pro-survival signalling has largely lost its function during ischaemia/reperfusion injury and the injury is serious, such that the effect of further inhibition of this pro-surviving pathway may not be obvious. The unchanged Akt activity and increased CTMP expression support this possible mechanism.

Several limitations of this study need to be mentioned. First, no significant differences were found between the groups in neurological score assessment. Exclusion due to inadequate rCBF decrease or death might explain the negative results, at least in part, another more sensitive neurological assessment system might be more suitable for this situation. Second, the use of a GSK3 fusion protein to examine Akt kinase activity might not reflect total Akt activity in vivo.

In summary, sevoflurane preconditioning inhibits elevated expression of CTMP triggered by brain ischaemia, and thus preserves Akt activity and its downstream pro-survival signalling in a transient focal cerebral ischaemic model. Development of specific inhibitors to CTMP might provide a possible treatment of cerebral ischaemic damage as in stroke.

Supplementary material

Supplementary material is available at British Journal of Anaesthesia online.
Authors’ contributions
H.D., L.X., and Y.C. were responsible for study conception and design. H.N. and L.T. participated in establishing animal and behavioural testing. Y.C. and L.T. performed molecular biological detection. J.D. and Y.Z. performed data analysis and interpretation. H.D. and L.X. obtained funding and provided administrative support. Y.C. and H.D. were responsible for drafting the manuscript. H.D. supervised the study.

Declaration of interest
None declared.

Funding
This work was supported in part by the State Key Program of National Natural Science Foundation of China (no. 30930091 to L.X.), the National Natural Science Foundation of China (no.81128005 to H.D., no.30972853 to H.D. and no.81401088 to Y.C.), and the Research Boosting Program of Xijing Hospital (no. XJZT13M10 to Y.C.).

References
4 McMurtrey RJ, Zuo Z. Isoflurane preconditioning and postconditioning in rat hippocampal neurons. Brain Res 2010; 1358: 184–90
11 Han BH, Holtzman DM. BDNF protects the neonatal brain from hypoxic–ischemic injury in vivo via the ERK pathway. J Neurosci 2000; 20: 5775–81
14 Hanada M, Feng J, Hemmings BA. Structure, regulation and function of PKB/AKT—a major therapeutic target. Biochim Biophys Acta 2004; 1697: 3–16
29 Li QF, Zhu YS, Jiang H. Isoflurane preconditioning activates HIF-1alpha, iNOS and Erk1/2 and protects against oxygen-glucose deprivation neuronal injury. Brain Res 2008; 1245: 26–35
31 Bedirli N, Bagriacik EU, Emmez H, Yilmaz G, Unal Y, Ozkose Z. Sevoflurane and isoflurane preconditioning provides neuroprotection by inhibition of apoptosis-related mRNA expression in a rat...
Role of CTMP in sevoflurane neuroprotection


35 Wang L, Traystman RJ, Murphy SJ. Inhalational anesthetics as preconditioning agents in ischemic brain. Curr Opin Pharmacol 2008; 8: 104 – 10


