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Activation of K_2P channel–TREK1 mediates the neuroprotection induced by sevoflurane preconditioning

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Editor's key points

- Volatile anaesthetic preconditioning can provide protection from ischaemia–reperfusion injury.
- Sevoflurane preconditioning reduced cell death, infarct size, and neurological injury in cellular and animal models of neuronal ischaemia.
- Knockdown of TREK-1 reduced sevoflurane-induced neuroprotection, indicating a role for this ion channel in this effect.

Background. Preconditioning with volatile anaesthetic agents induces tolerance to focal cerebral ischaemia, although the underlying mechanisms have not been clearly defined. The present study analyses whether TREK-1, a two-pore domain K^+ channel and target for volatile anaesthetics, plays a role in mediating neuroprotection by sevoflurane.

Methods. Differentiated SH-SY5Y cells were preconditioning with sevoflurane and challenged by oxygen–glucose deprivation (OGD). Cell viability and expression of caspase-3 and TREK-1 were evaluated. Rats that were preconditioned with sevoflurane were subjected to middle cerebral artery occlusion (MCAO), and the expression of TREK-1 protein and mRNA was analysed. Neurological scores were evaluated and infarction volume was examined.

Results. Sevoflurane preconditioning reduced cell death in differentiated SH-SY5Y cells challenged by OGD. Sevoflurane preconditioning reduced infarct volume and improved neurological outcome in rats subjected to MCAO. Sevoflurane preconditioning increased levels of TREK-1 mRNA and protein. Knockdown of TREK-1 significantly attenuated sevoflurane preconditioning-induced neuroprotective effects *in vitro* and *in vivo*.

Conclusions. Sevoflurane preconditioning-induced neuroprotective effects against transient cerebral ischaemic injuries involve TREK-1 channels. These results suggest a novel mechanism for sevoflurane preconditioning-induced tolerance to focal cerebral ischaemia.

Keywords: anaesthetics volatile, sevoflurane; brain, ischaemia; neuroprotection; preconditioning; TREK-1

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Stroke is a major cause of disability and is responsible for 40% of severely disabled adults.[1] Cerebral ischaemic events in the perioperative period increase mortality among surgical patients. The incidence of perioperative stroke is 0.08–0.7% in general surgery but up to 8–10% in heart valve surgery or aortic arch repair.[2] There is an unmet need for developing more effective and safer approaches for reducing the risk of cerebral ischaemic/reperfusion injury during the perioperative period.

Ischaemic preconditioning-induced neuronal tolerance to ischaemia was first reported by Kitagawa and colleagues[3] in 1990. However, ischaemic preconditioning is invasive and impractical for clinical practice. Several groups have found that pharmacological preconditioning methods such as volatile anaesthetics preconditioning can induce ischaemic tolerance in mice and rats.[4–6] Furthermore, volatile anaesthetic preconditioning has been used in coronary arterial surgery for cardiac protection in a rat model.[7] Volatile anaesthetic preconditioning is a promising strategy for perioperative neuroprotection that is less invasive, and sevoflurane preconditioning has been shown inducing tolerance against cerebral ischaemia insults in animal models.[8 9] However, the underlying mechanisms for its neuroprotective effects are unclear.

Two-pore domain background potassium channels (K_2P) are a diverse and highly regulated superfamily of ion channels that likely modulate membrane excitability in physiological functions.[10] As a subfamily of K_2P channels, TREK channels are predominantly expressed in the central nervous system.[11] TREK channels can be activated by membrane stretch, temperature, and internal acidosis, and are regulated by various pharmacological agents and cellular lipids, including volatile

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anaesthetics. Recent studies have suggested that the activation of TREK channels including TREK-1 mediates neuroprotection.[12] Moreover, researchers have demonstrated that TREK-1 plays an essential role for anaesthesia, neuroprotection, depression, and pain.[13] We hypothesized that TREK-1 plays a crucial role in mediating the neuroprotective effect against cerebral ischaemia afforded by sevoflurane preconditioning.

Methods

Animals and cells

All experimental procedures were carried out in accordance with the protocols approved by 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. SH-SY5Y cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Adult male Sprague–Dawley rats (280–320 g) were purchased from the Laboratory Animal Center of our university.

Experimental protocol

Role of TREK-1 in the neuroprotective effect of sevoflurane differentiated SH-SY5Y cells

SH-SY5Y human neuroblastoma cells were stimulated by all-*trans*-retinoic acid (RA) in cell culture for several days to stimulate differentiation into neurone-like cells, as identified by expressions of the neurone markers NeuN and βIII-tubulin. Differentiated cells were then subjected to various treatments. In the OGD group, cells were challenged by oxygen–glucose deprivation (OGD). In the Pre+OGD group, cells were exposed to 3.3 vol% sevoflurane for 2 h, and then after 1 h, they were subjected to OGD. In the siRNA group, small RNA interference (siRNA) was used to inhibit the expression of TREK-1 24 before sevoflurane preconditioning and OGD. In the siRNA-c group, control siRNA served as a reference to judge the effect of siRNA vector. Cells in the normal group were cultured without any treatments. After completion of treatments, cell viability was measured by the 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide (MTT) assay and caspase-3 expression was examined by western blot and immunofluorescence.

Role of TREK-1 in neuroprotection induced by sevoflurane in cerebral ischaemic rats

In order to investigate the effect of sevoflurane preconditioning against ischaemia–reperfusion injury, rats were randomly divided into three groups using an online randomization programme: sham, ischaemia (MCAO), and sevoflurane preconditioning plus ischaemia (Pre+MCAO) groups (n=8–9 each). Functional neurological outcomes were observed at 24, 48, 72 h, and 1 week after middle cerebral artery occlusion (MCAO) injury, and cerebral infarct volumes were measured at 72 h and 1 week. In addition, western blot and real-time polymerase chain reaction (PCR) analysis were used to detect levels of TREK-1 protein or in RNA at 4, 24, and 48 h after ischaemia–reperfusion injury in another set of animals.

In order to verify the role of TREK-1 in sevoflurane preconditioning-induced neuroprotection against ischaemia, we created an siRNA construct against TREK-1. The reliability of the siRNA was tested in normal rats before use. To examine the impact of siRNA on the neuroprotective effect of sevoflurane pretreatment, rats were randomly allocated to the following groups: sham, MCAO, sevoflurane preconditioning plus MCAO (Pre+MCAO), TREK siRNA plus sevoflurane preconditioning plus MCAO (siRNA+Pre+MCAO), and control siRNA plus sevoflurane preconditioning plus MCAO (siRNA-c+Pre+MCAO). The effect of siRNA on functional neurological outcomes was observed at 24, 48, and 72 after ischaemia, and cerebral infarct volume was compared at 72 h after ischaemia–reperfusion.

Cell culture and characterization

The human neuroblastoma cell line SH-SY5Y is often stimulated by RA to differentiate into neurone-like cells to model the responses of neurones.[14 15] Exponentially growing SH-SY5Y cells were grown in an airtight, integrated temperature-controlled cell culture chamber. Cells were kept in a 1:1 mixture of Ham's F12 nutrient and Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat inactivated fetal bovine serum, 0.28 μg ml^{-1} of gentamicin, and 250 μg ml^{-1} of amphotericin B, in a humidified atmosphere of 5% CO_2 in air at 37°C until 90% confluence was reached. Then, 10 μM of RA (1% of the total volume, R2625, Sigma-Aldrich, St Louis, MO, USA) was added into the medium to stimulate the cells to differentiate. Every 2–3 days, the culture medium was replaced. Expression of the neuronal markers NeuN and βIII-tublin was examined by western blot at 1, 4, 7, and 10 days during the differentiation process. Since the western blot results showed that the highest level of neuronal marker expressions occurred at 7 days after RA stimulation, cells were used at this time point for further experiments. These experiments included the localization of the above-mentioned markers and TREK-1 by immunofluorescence.

Sevoflurane preconditioning and OGD *in vitro*

Preconditioning conditions were achieved by incubation of the cells in 3.3 vol% sevoflurane with 5% CO_2 and 95% air for 2 h.[16 17] Cells were then placed in the incubator filled with 5% CO_2 95% air for 1 h before OGD.

To induce ischaemic injury, cells were subjected to OGD treatment. Cultures were first washed twice with DMEM without glucose and then put into pre-warmed OGD medium at 37°C for 120 min. The OGD buffer containing 154 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.3 mM $CaCl_2$, and 3.6 mM $NaHCO_3$ was bubbled with 95% air and 5% CO_2. OGD was terminated by returning to normal culture medium for an additional 24 h.

RNA interference

TREK-1 siRNA and negative control scrambled siRNAs were designed and purchased from Qiagen (Italy). The target sequence of the TREK1-siRNA used in this study was: