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Laboratory Investigation

LABORATORY INVESTIGATION

Activation of cannabinoid receptor 1 is involved in protection against mitochondrial dysfunction and cerebral ischaemic tolerance induced by isoflurane preconditioning

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Abstract

Background. Isoflurane preconditioning (IPC) induces cerebral ischaemic tolerance, but the mechanism remains poorly understood. The aim of this study was to determine changes in mitochondrial function in the brain after IPC, and whether the cannabinoid receptor 1 (CB1R) could be involved in the mechanism of mitochondrial protection mediated by IPC.
Methods. Adult male Sprague–Dawley rats were pretreated with isoflurane 2% for 1 h day^{-1}, for 5 days consecutively, and then subjected to 120 min right middle cerebral artery occlusion. Cannabinoid receptor 1 expression in the cellular and mitochondrial membrane was measured. The CB1R agonist HU-210 was administered alone, or the antagonists AM251 and SR141716A were given to the animals before each preconditioning. Neurological scores, infarct volume, apoptosis, and mitochondrial function were examined after middle cerebral artery occlusion.
Results. Expression of CB1R on cellular and mitochondrial membranes was increased 6 h after preconditioning. Both IPC and HU-210 administration before middle cerebral artery occlusion improved neurological outcomes and reduced infarct volume. Isoflurane preconditioning increased the expression of the anti-apoptotic proteins Bcl-2 and Bcl-X_L and reduced apoptosis in neurones. Isoflurane preconditioning and HU-210 also markedly preserved the activity of respiratory chain complexes, reduced mitochondrial radical generation, preserved mitochondrial membrane potential, and inhibited mitochondrial permeability transition pore opening. Cannabinoid receptor 1 antagonists abolished the improvement in mitochondrial function and the neuroprotective effects induced by IPC.
Conclusions. Our results indicate that IPC elicits brain ischaemic tolerance and mitochondrial protection by activating the CB1R, which provides a new mechanism for IPC-induced neuroprotection against cerebral ischaemia.

Key words: anaesthetics, cannabinoids; mitochondria; inhalation

Perioperative stroke during cardio-cerebral surgery results in high mortality and severe disability.[1] Volatile anaesthetics elicit tolerance in experimentally mediated ischaemia–reperfusion (I/R) cerebral damage.[2] Isoflurane is one of the most widely used volatile anaesthetic agents, and isoflurane preconditioning (IPC) results in neuroprotection against experimental brain

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Editor's key points

- Preconditioning with isoflurane (IPC) protects against ischaemia–reperfusion cerebral damage.
- The cannabinoid receptor CB1R modulates neuroprotection and may have a role in isoflurane-mediated effects through maintenance of mitochondrial function.
- Rats were subjected to middle cerebral artery occlusion plus IPC with and without CB1R agonist or antagonists.
- Cannabinoid receptor 1 agonists mimicked the protective effects of IPC, whereas antagonists reduced the protection.
- Neuroprotection by IPC involves the CB1R.

ischaemia.[3 4] However, the exact molecular and subcellular mechanisms underlying the neuroprotective actions of IPC are not yet clarified.

Mitochondria play a crucial role in generation of reactive oxygen species, iron homeostasis, and apoptotic signalling.[5] Mitochondrial dysfunction exacerbates I/R injury with activation of mitochondrial apoptotic cascades;[6–8] therefore, mitochondria could be a mechanistic target for the neuroprotective effect of preconditioning after brain ischaemic damage.[9–12] However, the mechanism of regulation of mitochondrial function by preconditioning requires further exploration.

Cannabinoid receptor 1 (CB1R) is a G-protein-coupled receptor, located in the neuronal plasma membrane, which modulates neuronal activity, synaptic plasticity, and cell metabolism.[13] Accumulating evidence has demonstrated a substantial role for CB1R in neuroprotection[14 15] and in the brain ischaemic tolerance induced by ischaemic preconditioning and electro-acupuncture.[16 17] Inhibition of CB1R using antagonists or a knockout strategy blocked neuroprotection both *in vivo* and *in vitro*.[18–20] Recent investigations showed that CB1R was also expressed in neuronal mitochondrial membranes (mtCB1R), regulating mitochondrial respiration, energy metabolism, and other functions.[21 22] However, the involvement of mtCB1R in the neuroprotection induced by IPC is still unknown.

In the present study, we used a transient focal cerebral I/R model in rats and Ca^{2+}-induced injury in purified intact mitochondria to investigate the role of mtCB1R in the neuroprotection induced by IPC both *in vitro* and *in vivo*.

Methods

Animals

All animal-related procedures were approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University (Xi'an, China), in accordance with the US National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and relevant aspects of the ARRIVE guidelines.

Male Sprague–Dawley rats (8–10 weeks old), weighing 270–320 g, were used. The animals were housed with a 12 h–12 h light–dark cycle, at 20–25 °C, and 60% humidity. Unrestricted water and food were available.

Experimental protocols

Experiment 1

The long-term neuroprotective effect of IPC was determined. Rats (n=8 per group) were divided into three groups as follows: sham, middle cerebral artery occlusion (MCAO), and isoflurane preconditioning (ISO group). Seven days after reperfusion, neurological outcome and infarct volume were determined. Full details are provided in the Supplementary file S1.

Experiment 2

Cannabinoid receptor 1 expression after IPC was examined. Rats were allocated to two groups without MCAO: oxygen control or isoflurane preconditioning (IPC). Cannabinoid receptor 1 protein expression was evaluated by western blot in control rats and at different time points (2, 6, 12, 24, and 48 h, n=6 at each time point) after IPC. Cannabinoid receptor 1 immunofluorescence staining was also undertaken in both control and IPC groups at 6 h (n=3). In addition, at the same time point as the last IPC, CB1R expression in plasma membrane and mitochondria was assessed in isolated mitochondria by western blot (n=6) and immunogold electron microscopy (n=3).

Experiment 3

To investigate the role of CB1R in IPC-mediated neuroprotection and amelioration of mitochondrial dysfunction, the CB1R agonist HU-210 was pre-administered based on a previous study.[21] Twenty-four rats were divided into three groups (sham, MCAO+vehicle, and MCAO+HU-210), and neurological behaviour, infarct volume, and apoptosis [terminal deoxynucleotidyl transferase-mediated 2′-deoxyuridine 5′-triphosphate nick-end labelling (TUNEL) staining] were measured. Additionally, two CB1R antagonists, AM251 and SR141716, were administrated as described previously.[16 23] Forty-eight rats were divided into the following six groups: sham, MCAO, ISO (IPC+MCAO), ISO+AM251 (IPC+MCAO+AM251), ISO+vehicle (IPC+MCAO+vehicle), and ISO+SR141716 (IPC+MCAO+SR141716). Neurological outcome, cerebral infarct volume, and apoptosis level (TUNEL staining, prosurvival protein expression, and cleaved caspase-3 concentrations) were evaluated 72 h after reperfusion. The activity of mitochondrial respiratory chain complexes was also measured 4 and 24 h after reperfusion.

Experiment 4

Citrate synthase, mitochondria membrane potential (MMP), mitochondrial permeability transition pore (mPTP) opening, and total radical production were measured in isolated mitochondria. Intact brain mitochondria were divided into the following seven groups: sham; control, where 200 μM $CaCl_2$ was added to induce mitochondrial dysfunction; ISO, where mitochondria were pre-incubated with isoflurane (0.5 mM) for 15 min, and then the suspension was diluted in isolation buffer and centrifuged at 8000***g*** for 10 min; ISO+AM251 and ISO+SR141716A, where the CB1R antagonists (AM251 or SR141716A) were added with Ca^{2+} after isoflurane exposure; ISO+vehicle, vehicle was added with Ca^{2+} after isoflurane exposure; and HU-210, where HU-210 (6 μM) was added 30 min before Ca^{2+} supplementation. Twenty minutes after Ca^{2+} treatment, citrate synthase, MMP, mitochondrial swelling, and total radical generation were measured.

Determination of activity of respiratory chain complexes

Evaluation of all respiratory chain enzyme activities was undertaken spectrophotometrically at 30 °C according to methods reported previously.[24] In brief, NADH-ubiquinone oxidoreductase (complex I) activity was measured as the rate of NADH-dependent ferricyanide reduction. Succinate-2,6-dichloroindophenol (DCIP)-oxidoreductase (complex II) was measured as the reduction of 2,6-DCIP. Coenzyme Q-cytochrome *c* reductase (complex III)

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