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Hypothermic preconditioning but not ketamine reduces oxygen and glucose deprivation induced neuronal injury correlated with downregulation of COX-2 expression in mouse hippocampal slices

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ABSTRACT

Hypothermic preconditioning is an effective treatment for limiting ischemic injury, but the mechanism is poorly understood. This study was aimed to explore the effect of hypothermic and ketamine preconditioning on oxygen and glucose deprivation (OGD) induced neuronal injury in mouse hippocampal slices, and to investigate its possible mechanism. The population spike (PS) was recorded in the CA1 region of mouse hippocampal slices using extracellular recordings, Na^+/K^+ ATPase activity in slices was determined by spectrophotometer and the expression of Cyclooxygenase-2 (COX-2) was measured by Western blot. Ten min of OGD induced a poor recovery of PS in slices after reoxygenation. Hypothermic (33 °C) preconditioning delayed the appearance of transient recovery (TR) of PS and improved the recovery amplitude of PS after reoxygenation. Hypothermic preconditioning also decreased the expression of COX-2 and increased Na^+/K^+ ATPase activity in slices. Pretreatment of ketamine, a non-competitive NMDA receptor antagonist at a subanesthetic dose has no effect on OGD induced neuronal injury. Moreover, the protection of hypothermic preconditioning was not added by ketamine. The downregulation of COX-2 expression and the increase of Na^+/K^+ ATPase activity may be associated with the effectiveness of hypothermic preconditioning in increasing tolerance to an OGD insult.

1. Introduction

Hypothermic preconditioning has been reported to safely improve neurologic outcomes in rodent models of cerebral ischemia, based on laboratory investigations in vivo and in vitro,[1,2] but the underlying mechanism of hypothermic preconditioning remains unclear. Cyclooxygenase (COX), known as prostaglandin-endoperoxide synthase, is an enzyme that is responsible for the generation of prostanoids, including thromboxane and prostaglandins.[3,4] COX has two isoforms (COX-1 and COX-2), while COX-2 is expressed widely in some organs, such as brain, kidney and reproductive organs.[5,6] COX-2 is thought to play a role in neuroinflammation and excitotoxicity, which is important component in the progression of ischemic injury.[7] Therefore, COX-2 is an attractive target for the treatment of ischemic injury.

In the previous studies, there were discrepant results about the effect of hypothermia on COX-2 protein after brain ischemia. Xiang et al[8] reported that intra-ischemic mild hypothermia significantly reduced neuronal damage in both wild type mice and transgenic mice with neuronal overexpression of human COX-2. However, Yamashita et al[9] found that hypothermic, but not normothermic ischemia increased COX-2 immunoreactive granule cells in rat dentate gyrus after ischemia. Similar changes in microglial areas in the dentate gyrus and CA1 region were also reported.[10] Although some studies suggest that COX-2 activity contributes to neuronal death after ischemia,[11,12] it may be relatively resistant to ischemic insult, which is not necessarily followed by cell death.[13] It is possible that COX-2 plays differential roles at different stages of the pathogenesis after ischemia.[14] The relationship between the expression of COX-2 and hypothermic preconditioning is worth further study.

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The anesthetics, such as propofol, isoflurane and sevoflurane which are frequently used in the perioperative setting, are suggested to produce the protective effects.[15–17] There are different effects between the anesthetics applied before and during ischemia or only before ischemia. Ketamine is a dissociative anesthetic known to be a non-competitive antagonist of NMDA receptor. When administration before and during brain ischemia, ketamine at a subanesthetic or anesthetic dose could improve synaptic transmission and attenuate neuronal damage.[18,19] In contrast, some studies showed that ketamine failed to protect against ischemic neuronal injury.[20,21] Moreover, ketamine may block ischemic preconditioning mediated by activation of the NMDA receptor.[22] However, it is still unknown whether the administration of subanesthetic ketamine only before ischemia will provide the protection against neuronal injury.

The hippocampal slice preparation is very useful in vitro model for studying the protective mechanism of drugs or hypothermia on cerebral ischemia, especially be combined with the biochemical and electrophysiological methods.[23,24] In this study, we tested the effects of ketamine and hypothermic preconditioning on OGD-induced loss of synaptic transmission in mouse hippocampal slices, and its relative mechanism of COX-2 expression. We also investigated whether ketamine could provide added protection to hypothermic preconditioning.

2. Materials and methods

2.1. Animal and drugs

Adult male Kunming mice (20–24 g) were employed in the present studies. Mice were housed under a 12-h light/12-h dark cycle regime, with free access to food and water. The animals were provided by Experimental Animal Center of Xuzhou Medical University. All experimental protocols were approved by the Animal Care and Use Committee of the college and were in accordance with the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 80–23, revised 1996). Ketamine was purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Slices preparation

Animals were killed by decapitation, and their brains were rapidly removed and immersed in an ice-cold artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl (124), KCl (5), $CaCl_2$ (2.5), $MgSO_4$ (1.3), KH_2PO_4 (1.25), $NaHCO_3$ (24) and D-glucose (10), which was bubbled with a mixture of 95% O_2/5% CO_2. After dissection, the hippocampus was cut into transverse slices (450 μm thick) using McIlwain tissue chopper (Campden Instruments, UK). Slices were kept in a holding chamber at room temperature for 1–6 h. During the experiment, 8–10 slices were transferred to a small circular nylon mesh net located in a 3.5 ml submerged chamber with oxygenated ACSF. The slices were covered by a second nylon mesh net and superfused at 3 ml/min. The baseline temperature of the perfusing medium was maintained at 37 °C.

2.3. Temperature manipulation

During the hypothermic experiment, the baseline temperature of the perfusing medium was decreased (0.5 °C/min) from 37 °C to 33 °C, and maintained for 20 min at 33 °C, then the temperature was rewarmed to baseline. The temperature was regulated with a thermostatic controller (SWKY-1, Nanbei Instruments, China), and continuously monitored by a thermistor probe situated within the chamber adjacent to the slices.

2.4. Electrophysiological experiments

Electrophysiological responses were recorded from one of the slices in the chamber. A bipolar stainless-steel stimulating electrode (10 MΩ) was placed in stratum radiatum, and the stimulation pulse (2.94 ± 0.2 V, 0.2 ms pulse duration) was delivered at 0.05 Hz. Glass microelectrode (2 M NaCl, 2–5 MΩ) was placed in stratum pyramidale to record population spike (PS). Responses were filtered (with band-pass filters at 0.5–10 kHz), sampled (10 kHz), and stored by a Macintosh computer with MacLab (AD Instruments). After stabilization of responses, an input–output curve was constructed, and stimulation intensity was adjusted to evoke 60% of maximum amplitude of PS. Some slices that showed a change in the amplitude of PS >20% during 10-min monitoring were discarded. In 6 out of 45 slices, the amplitude of PS was considered unstable during the preliminary observation period were discarded.

2.5. OGD and preconditioning

OGD was induced by superfusing slices with glucose-free ACSF equilibrated with 95% N_2 and 5% CO_2. Mice were divided into control, OGD, Ketamie (K), hypothermia (H) and hypothermia + ketamine (H + K) groups. The slices were pretreated for 20 min with hypothermia (33 °C) or subanesthetic ketamine (10 μM) in slices with normal ACSF, following rewarmed or washed out for 10 min and then subjected to 10 min of OGD. After OGD, the slices were reoxygenated at least 60 min with ACSF equilibrated with 95% O_2 and 5% CO_2. In the experiments, the recovery amplitude of PS was monitored from one of the slices in the chamber, the other slices were removed from the chamber immediately at 60 min after reoxygenation for the determination of Na^+/K^+ ATPase activity and COX-2 expression.

2.6. Western blot

Some slices were rapidly frozen in liquid nitrogen or stored at −80 °C for subsequent procedures. The frozen tissues were directly homogenized in a lysis buffer containing a cocktail of protease inhibitors. At the end of 15-min centrifugation at 12,000 rpm at 4 °C, the supernatant was collected for Western blot. 30 μg of protein of each sample was separated by 10% SDS-PAGE and then electrotransferred onto nitrocellulose membranes. Membranes were incubated with rabbit polyclonal anti-COX-2 antibody (1:1000, Santa Cruz Biotechnology) or anti-β-actin (1:1000, Santa Cruz Biotechnology) primary antibody overnight at 4 °C. Anti-rabbit secondary antibody (1:1000, ZSGB-Bio, China) was incubated for 2 h at room temperature. The densitometry analysis of signal intensity was performed using ImageJ software. The relative levels of protein in the hippocampus were normalized to β-actin.

2.7. Na^+/K^+ ATPase activity

To measure Na^+/K^+ ATPase activity, some slice was made into 1% homogenates in saline. The homogenates were then centrifuged at 3000 rpm and 4 °C for 10 min. Na^+/K^+ ATPase activity in the supernatant was evaluated spectrophotometrically with the Testing Kit (Jiancheng Bioengineering, Nanjing, China) following the manufacturer's protocol. The activity of Na^+/K^+ ATPase was determined by Spectrophotometry using ATP as substrate; thus, 1 μmol inorganic phosphorus generated by ATPase enzyme decomposition is equal to one ATPase enzyme activity unit (μmol Pi h^{-1} mg^{-1}