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Involvement of the mitochondrial ATP-sensitive potassium channel in the neuroprotective effect of hyperbaric oxygenation after cerebral ischemia

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Abstract

In the present study, we investigated whether activation of mitochondrial ATP-sensitive potassium channel is involved in the neuroprotective effect offered by early hyperbaric oxygenation after cerebral ischemia. The selective mitochondrial ATP-sensitive potassium channel antagonist 5-hydroxydecanoate was infused intracerebroventricularly before hyperbaric oxygenation treatment initiated 3 h after middle cerebral artery occlusion for 90 min. Neurological status was evaluated and brains were removed for the measurement of infarct size and immunohistochemical evaluation of apoptosis 24 h after middle cerebral artery occlusion. Early hyperbaric oxygenation treatment improved neurologic deficits and reduced infarct volume, while these effects were reversed by the administration of 5-hydroxydecanoate. Furthermore, early hyperbaric oxygenation significantly decreased the number of apoptotic cells in the peri-infarct cortex 24 h after ischemic insult and this effect was also blocked by 5-hydroxydecanoate. The present findings suggest that early hyperbaric oxygenation therapy prevents apoptosis and promotes neurologic functional recovery after focal cerebral ischemia, and the opening of mitochondrial ATP-sensitive potassium channel plays a role in this antiapoptotic effect of early hyperbaric oxygenation.

Keywords: Hyperbaric oxygenation; Ischemia; Middle cerebral artery occlusion; Mitochondria; Potassium channels; Rats

1. Introduction

By increasing the oxygen content of blood and improving tissue oxygenation, hyperbaric oxygenation (HBO) has been implicated as an attractive procedure for use in cerebral ischemia. A long-lasting neuroprotective effect of early HBO treatment during brain ischemic injury has been demonstrated in several studies [16,23,32,38]. The investigation of therapeutic window for the use of HBO indicates that HBO is highly efficient in reducing ischemic injury in transient focal ischemia within the first 6 h [4,28]. However, HBO is not currently used in acute stroke management, partially due to the insufficient information of its molecular mechanism.

In the past, several studies have addressed the question of whether HBO treatment might affect the recovery of mitochondrial function in cerebral ischemia, since HBO therapy, such a hyperoxic condition, will undoubtedly affect cerebral energy metabolism, whereas mitochondria plays critical roles in cerebral energy metabolism and mediating cellular responses to ischemic/hypoxic stress. In fact, most of the researches proved the change of aerobic metabolic function such as the activity of respiratory chain complex and redox potential influenced by HBO therapy [11,12]. However, little is known about the contribution of mitochondria in the initiation and regulation of processes leading to reduction of ischemic injury after HBO treatment.

The mitochondrial ATP-sensitive potassium channel (mitoK_{ATP}) is localized in the inner membrane of mitochondria and regulates the function of the mitochondria. It was demonstrated that selective opening of the mitoK_{ATP} has neuroprotective effect against ischemia-reperfusion injury in the rat brain and these protective effects can be prevented by 5-hydroxydecanoate (5-HD), a selective antagonist of mitoK_{ATP} [35]. Furthermore, increasing evidences indicate that activation of mitoK_{ATP} can directly protect cerebellar neurons from apoptosis and modulate neuronal survival under

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ischemic conditions by a procedure involving suppression of Bax translocation and cytochrome *c* release from mitochondria to cytoplasm [1,26,36]. It was also demonstrated that opening of the mitoK$_{ATP}$ followed by mitochondrial swelling could preserve the normal mitochondrial oxygen consumption rate and improve mitochondrial ATP production and/or handling during hypoxia [39,20]. Taken together, we thus were prompted to investigate whether increasing oxygen levels by HBO offers neuroprotection, at least partially by activation of mitoK$_{ATP}$ in the brain and inhibition of apoptotic processes in peri-infarct tissue. We tested whether the selective mitoK$_{ATP}$ antagonist 5-HD or glibenclamide (both sarcolemmal and mitoK$_{ATP}$ blocker) infused intracerebroventricularly (ICV) before HBO treatment would reverse the recovery of neurologic outcome, infarct size and abolish the antiapoptotic effect of HBO treatment after transient middle cerebral artery occlusion (MCAO) in rats. DNA fragmentation assessed with terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL), the cleavage fragment of the caspase-3 and caspase-9 in the peri-infarct cortex was quantified as indicators of apoptosis.

2. Materials and methods

Male Sprague–Dawley rats weighing 200 g were obtained from Charles River (Sulzfeld, Germany) and Zhejiang Academy of Medical Sciences (China) and kept under controlled conditions. The local ethics committee from Kiel and Zhejiang University approved all experimental protocols. Chloral hydrate (400 mg/kg body weight) injected intraperitoneally was used as anesthetic for all surgical procedures.

2.1. Study design

Animals were randomly assigned to the following groups ($n = 10$ in each group): group A (control group), ICV administration of saline, room air; group B, ICV administration of saline, HBO treatment; group C, ICV administration of 5-HD, HBO treatment; group D, ICV administration of 5-HD, room air; group E, ICV administration of glibenclamide, HBO treatment.

2.2. Middle cerebral artery occlusion (MCAO) with reperfusion

The intraluminal occlusion of the middle cerebral artery (MCA) for 90 min with subsequent reperfusion was used [22]. Regional cerebral blood flow (rCBF) was continuously monitored at one point (1 mm posterior to the bregma, 5 mm from the midline) on the surface of each hemisphere in the supply territory of the MCA before, during, and after MCAO by laser-Doppler-flowmetry (Periflux system 5000) [34]. rCBF measurements were performed to assess the degree of MCAO, as we used previously. Abrupt reduction in rCBF by approximately 75–90% indicated a successful occlusion of the MCA. Rats in which the ipsilateral blood flow during ischemia was not reduced to less than 30% of baseline during the first 30 min of occlusion, or in which a premature increase in the ipsilateral blood flow was recorded, were excluded from the experiments. Body temperature was maintained at 37 °C with a heating pad. Physiological parameters (rectal temperature, arterial pH, PCO_2, PO_2, hemoglobin, hematocrit, glucose, potassium, sodium, calcium, and chloride) were monitored in each group throughout the studies as previously reported.

2.3. Intracerebroventricular (ICV) administration of drugs

The head was stabilized in a stereotactic frame (David Kopf Instruments) and a hole was made in the skull. 5-Hydroxydecanoate (5-HD) (Sigma; 100 mM, dissolved in 10 μl normal saline) or glibenclamide (Sigma; 0.1 mg/kg, dissolved in 20 μl dimethyl sulfoxide) or saline (10 μl) was infused into the right lateral ventricle (0.6 mm caudal to bregma, 1.3 mm lateral to the midline, and 5.0 mm vertical from the skull surface) via polyethylene tubing to a glass microlitre syringe (SGE, Germany) 15 min before HBO treatment. Immediately after the ICV administration of drugs, wounds were closed.

2.4. Hyperbaric oxygenation (HBO)

HBO was performed in an experimental pressure chamber. During HBO administration, animals could be observed through the transparent acrylic glass. HBO was administered at a pressure of 3 atm absolute (3 ATA) for 1 h with 100% oxygen, starting at 3 h after MCAO. Compression and decompression were achieved within 5 min. The controls (groups A and D) received the same dose of anesthesia corresponding to the time points of HBO.

2.5. Evaluation of neurological deficits

According to the Garcia neurological grading systems, the neurological status of each rat was evaluated 24 h after MCAO by a blinded observer [15].

2.6. Tissue processing

Twenty-four hours after MCAO, rats were deeply anesthetized and intracardially perfused with phosphate-buffered saline (PBS) (pH 7.4) followed by 4% paraformaldehyde. The brains were removed, postfixed overnight, and subsequently cryoprotected in 30% sucrose for 72 h at 4 °C. Coronal sections (40 μm) were cut in a cryostat (from bregma +3.7 to −6.7 mm). Every 20th slice was used for the determination of infarct size. Three consecutive slices obtained at the level of the anterior commissure (bregma ±0) were used for the determination of apoptosis (staining for TUNEL, activated caspase-3, -9).

2.7. Measurement of infarct volume

In total, 14 coronal brain sections were stained with cresyl violet. Sections were digitalized with the use of a scanner and analyzed by a blinded investigator using Image J (NIH, USA). To eliminate brain edema, the corrected infarct volume was calculated as described in detail by Schäbitz et al. [33].

2.8. Immunohistochemical detection of apoptosis

DNA fragmentation assessed with TUNEL, the cleavage fragment of the caspase-3 and caspase-9 in the peri-infarct cortex was investigated as indicators of apoptosis in immunohistochemical studies. TUNEL staining was carried out using an apoptosis detection kit according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). Only strongly labeled TUNEL-positive cells were considered as apoptotic, whereas lightly stained cells suggesting necrosis were not evaluated. Brain sections to be stained were incubated with the primary antibody overnight at 4 °C followed by biotinylated secondary antibody (anti-rabbit, Vector Laboratories Inc.) and the avidin-biotin complex peroxidase method (Vectastain, Vector Laboratories, Inc.). The following primary antibodies were used: rabbit anti-rat cleaved caspase-3 antibody (Cell Signaling Technology, dilution 1:100), rabbit anti-rat cleaved caspase-9 antibody (Cell Signaling Technology, dilution 1:100). All the procedures were conducted at room temperature. The brain sections were then mounted, air-dried, dehydrated, coverslipped, and observed under a microscope. As a negative control, a control staining with omission of primary antibody was performed and showed no specific staining (data not shown).

2.9. Morphometric studies

Coronal sections through the brain at the level of the anterior commissure were used for the immunohistochemical analysis of apoptosis. Three random and non-overlapping areas (0.125 mm^2 per area) were chosen in the boundary zone of the ischemic core in the frontoparietal cortex, as described in detail previously [27,41]. The quantification of positively stained cells was carried out using Leica image analyzing software (Leica Qwin).