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Molecular Brain Research 133 (2005) 87–94
MOLECULAR BRAIN RESEARCH
www.elsevier.com/locate/molbrainres

Research report

Activation of ATP-sensitive potassium channels prevents the cleavage of cytosolic μ-calpain and abrogates the elevation of nuclear c-Fos and c-Jun expressions after hypoxic–ischemia in neonatal rat brain

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Accepted 29 September 2004
Available online 2 November 2004

Abstract

The purpose of this study was to determine whether activation of ATP-sensitive K^+ (K_{ATP}) channels with diazoxide (DIZ) is able to prevent the cleavage of cytosolic μ-calpain and abrogate the elevation of nuclear c-Fos and c-Jun protein (c-Fos, c-Jun) expressions after hypoxic–ischemia (HI) in brain. The model of hypoxic–ischemic brain injury (HIBI) was made in the 7-day-old Sprague–Dawley (SD) rats by left carotid arterial ligation and hypoxia (8% oxygen). DIZ was injected into the left lateral ventricle (5 μl, 1 mg/ml) before or post-hypoxic–ischemia (HI) insults. Western blot and computer image processing were used to detect the integrated density of nuclear c-Fos and c-Jun at 4 h and cleavage of cytosolic μ-calpain at 24 h after HI insults from cerebral cortical and hippocampal samples. Compared with HI controls (c-Fos=30.37±7.39 from cortical samples, 58.61±3.64 from hippocampal samples; c-Jun=52.48±14.23 from cortical samples, 35.55±4.73 from hippocampal samples), there was a significant down-regulation of c-Fos and c-Jun expressions from cortical and hippocampal samples in rats treated with DIZ before (c-Fos=11.10±4.64 from cortical samples, 4.82±3.38 from hippocampal samples; c-Jun=19.01±5.29 from cortical samples, 35.55±4.73 from hippocampal samples) or post- (c-Fos=18.81±7.93 from cortical samples, 11.33±7.05 from hippocampal samples; c-Jun=24.64±10.01 from cortical samples, 19.75±3.47 from hippocampal samples) HI insults. Furthermore, the ratio of 76 kD/80 kD of μ-calpain was down-regulated from cortical and hippocampal samples in rats treated with DIZ before or post-HI insults, demonstrating a significant difference compared with that observed in HI controls. Finally, the increase in DNA fragments caused by the HI injury was decreased or eliminated by the treatment with DIZ. These data suggests that activation of K_{ATP} channels by DIZ reduces the degree of μ-calpain proteolysis, and c-Fos and c-Jun expressions in immature brain may contribute to the neuroprotection of K_{ATP} channel openers against HIBI.

Theme: Excitable membranes and synaptic transmission
Topic: Potassium channel physiology, pharmacology, and modulation

Keywords: Mitochondrial ATP-sensitive K^+ channel; μ-Calpain; Hypoxic–ischemic brain injury; c-Fos; c-Jun

1. Introduction

Hypoxia–ischemia (HI)-induced encephalopathy is a frequent cause of death and disability in children [24]. The mechanisms involved in mediating neuronal cell death in the neonate are complex and poorly understood. Nonetheless, it appears that strategies directed towards preserving mitochondrial function in adults are able to protect various organs and tissues against ischemic injury [8]. For example, ischemic preconditioning, which activates ATP-sensitive potassium (K_{ATP}) channels in mitochondria (mK_{ATP}), or pharmacological activation of plasma membrane K_{ATP}

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doi:10.1016/j.molbrainres.2004.09.020

(surface K_{ATP}) or mK_{ATP} channels with drugs such as diazoxide (DIZ) are able to limit ischemia-induced damage and cell death in the myocardium [26]. However, little is known about potential protective effects of surface K_{ATP} or mK_{ATP} channels activation in brain. The purpose of this study was to determine whether activation of surface K_{ATP} or mK_{ATP} channels with DIZ is able to prevent the cleavage of cytosolic μ-calpain and abrogate the elevation of nuclear c-Fos and c-Jun protein (c-Fos, c-Jun) expression after HI in 7-day-old rat brain.

2. Materials and methods

2.1. Animals and experiment protocols

Rats were obtained from the Experimental Animal Center of Zhejiang Medical Academy of Science. Procedures involving animals and their care were conducted in accord with our institutional guidelines that comply with international laws and policies (*NIH Guide for the Care and Use of Laboratory Animals*, NIH Publication No. 85-23, 1985).

Hypoxic–ischemic brain injury (HIBI) was produced as previously described by Rice et al. [18]. In brief, after the rats were anesthetized with ether inhalation, the left carotid artery was isolated, doubly ligated, and transected. After recovery in an incubator (37 °C, 2 h), the rats were exposed to a 8% O_2 and 92% N_2 gas mixture for 2 h in a glass chamber (35±1 °C) equipped with an inlet and an outlet for gases at a flow of 5 L/min.

All experiments were performed on 7-day-old Sprague–Dawley (SD) rats of either sex (weighing 11–12 g). Series 1 were designed to achieve a dose–response relationship of DIZ preconditioning on the c-Fos expressions and the blood sugar levels. Eighteen rats were randomized to be treated with 1, 3, 5, 7, and 9 μl DIZ (Sigma, 1 mg/ml) or vehicle, and other 18 rats were randomized to be treated with 0.1, 0.5, 1, 2 and 3 mg/ml DIZ or vehicle in a 5-μl volume 1 h prior to HI (three rats in each group). DIZ were dissolved in artificial cerebrospinal fluid (ACSF) (NaCl 124 mmol/L, KCl 1.8 mmol/L, $MgSO_4$ 1.8 mmol/L, $CaCl_2$ 1.6 mmol/L, NaH_2PO_4 1.25 mmol/L, D-glucose 10 mmol/L, and $NaHCO_3$ 26 mmol/L, pH 7.4) and diluted to the demanded concentration for administration to rats. DIZ was injected into the left lateral ventricle (icv) (1.5 mm lateral, 2 mm prior, and 2 mm ventral from lambdoid suture) at the rate of 1–2 μl/min. The blood sugar level from tail blood samples (100 μl) in hypoxic–ischemic rats was examined at 1 h after HI by using testing paper and its matching instrument (Glucotrend, Roche). Four hours after HI, rats were anesthetized with ether inhalation, and the brains were removed quickly, and the ischemic frontal cortex and hippocampus were carefully isolated (within 2 min on ice) and immediately frozen in liquid nitrogen and then stored at −80 °C until the time of assay.

Series 2 was aimed to determine if the administration with DIZ was able to prevent the cleavage of cytosolic μ-calpain and abrogate the elevation of nuclear c-Fos and c-Jun expression after HI (see Fig. 1). Forty-eight animals were divided into eight groups (six rats in each group) at random: normal control groups 1 and 2, HI control groups 1 and 2, DIZ preconditioning groups 1 and 2, and DIZ administration groups 1 and 2. Normal control groups 1 and 2 did not receive any procedure. Rats of DIZ preconditioning groups 1 and 2 received an injection with 5 μl DIZ (1 mg/ml) into the left lateral ventricle (icv) at the rate of 1–2 μl/min 1 h prior to HI insults, as well as immediately post-hypoxia insults in DIZ administration groups 1 and 2. While in HI control groups 1 and 2, rats were treated with same volume of ACSF. Animals of HI control group 1, DIZ preconditioning group 1, and DIZ administration group 1 were killed by decapitation at 4-h post-HI insults (those of normal control group 1 were sacrificed at the same time), as well as at 24-h post-HI insults for those of other groups. The samples were collected and stored as mentioned above.

Series 3 were to observe the effects of DIZ on the DNA fragmentation at 3 days after HI. Twenty-four rats were divided into four groups (six rats in each group) at random: normal group, HI group, DIZ preconditioning group, and DIZ administration group. Normal group did not receive any procedure. Rats of DIZ preconditioning and administration groups received an injection with 5 μl DIZ (1 mg/ml) into the left lateral ventricle (icv) 1 h prior to and immediately post-HI insults, respectively. While in HI group, rats were treated with same volume of ACSF.

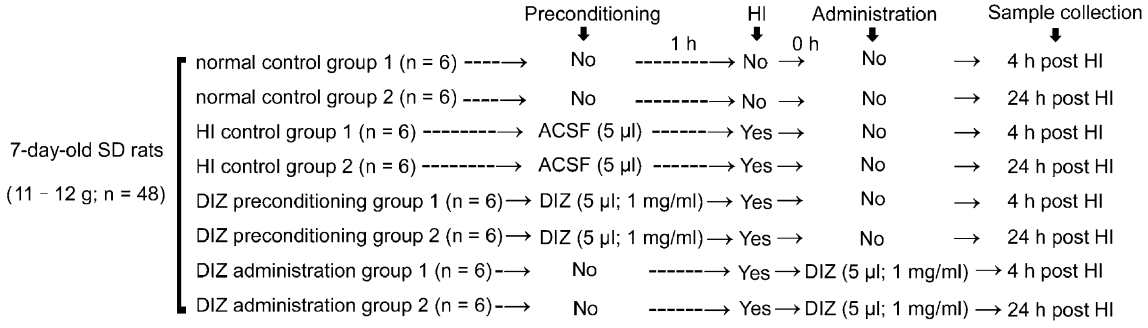

Fig. 1. Experimental protocols of series 2. DIZ—diazoxide, HI—hypoxia–ischemia.