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Effect of ischemic preconditioning on cerebral blood flow after subsequent lethal ischemia in gerbils

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Abstract

Ischemic tolerance, the phenomenon where a sublethal ischemic preconditioning protects the brain against a subsequent lethal ischemia, has been widely studied. Studies have been done on cerebral blood flow levels prior to the lethal ischemia, but the hemodynamic pattern after global ischemia with ischemic preconditioning has not been reported. Sequential changes in regional cerebral blood flow (rCBF) in gerbil hippocampus after 5 min global ischemia with or without 2 min ischemic preconditioning were studied to determine if ischemic preconditioning affects rCBF. Four different treatments were given: (1) sham-operated, (2) 2 min ischemia, (3) non-preconditioned, and (4) preconditioned. Groups (1) and (2) (both groups $n=5$) were given a 24-h recovery period and the rCBF was measured for baseline values. 24 h after sham-operation (3) and 2 min ischemia (4), gerbils were subjected to 5 min ischemia followed by 1 h, 6 h, 1-day or 7-day reperfusion periods (all groups $n=5$). Although no regional difference was observed in the recovery pattern of rCBF, the values of rCBF were significantly higher in the preconditioned group throughout whole brain regions including hippocampus. These results indicate that ischemic preconditioning facilitated the recovery of rCBF after 5 min global ischemia. It needs further study to determine whether the protecting effects of preconditioning relate to the early recovery of rCBF or not. However, our results could be interpreted that the early recovery of rCBF may lead to benefits for cell survival in the CA1 neuron, probably facilitating other protecting mechanisms.

Keywords: Autoradiography; Gerbil; Global ischemia; Hippocampus; Ischemic tolerance; Regional cerebral blood flow (rCBF)

Introduction

Ischemic tolerance is the phenomenon whereby ischemic preconditioning protects against a subsequent lethal ischemia. Ischemic tolerance was first demonstrated in the heart (Murry et al., 1986) and has been described in several organs including brain, kidney (Toosy et al., 1999), and liver (Peralta et al., 1997; Yin et al., 1998). In gerbil brain, ischemic preconditioning has been shown to protect hippocampal CA1 pyramidal cells from subsequent lethal ischemia (Kirino et al., 1991). Previous reports suggest that heat shock proteins (Kirino et al., 1991; Kitagawa et al., 1991; Nowak, 1985), immediate early genes (Ikeda et al., 1994; Sommer et al., 1995), antioxidant enzyme (Kato et al., 1995; Ohtsuki et al., 1992), antiapoptotic oncogene (Kitagawa et al., 1998; Shimazaki et al., 1994), interleukin-1β (Barone et al., 1998; Ohtsuki et al., 1996), and adenosine (Kawahara et al., 1998; Hiraide et al., 2001) might be involved in the development of ischemic tolerance. It has also been reported that ischemic preconditioning did not affect the energy metabolism or mitochondrial enzyme activity after 5 min global ischemia (Katayama et al., 1997). The protective mechanism of ischemic preconditioning are reported to involve intracellular signal transduction pathway including endoplasmic reticulum and DNA repairing function (Saito et al., 2004; Hayashi et al., 2003; Sugawara et al., 2001). The relationship between preconditioning, induced by spreading depression, and the rCBF just before focal ischemia was reported to be relate to protective mechanisms of ischemic tolerance (Otori et al., 2003). However, the precise mechanism of ischemic tolerance is still unclear.

Chen et al. (1996), using a focal ischemia model, reported that ischemic preconditioning does not affect rCBF in the tolerant regions before or during subsequent ischemia. However, there have been no studies on whether ischemic precondi-

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tioning affects rCBF in gerbil after subsequent lethal ischemia. The purpose of this study is to determine the effect of ischemic preconditioning on the chronological changes in rCBF after 5 min lethal ischemia, using an autoradiography technique.

Material and methods

Animals groups and preparation

This experiment was conducted in accordance with the Guidelines for Animal Experimentation of the Nippon Medical School. Male Mongolian gerbils (Sankyo Labo Service, Tokyo, Japan) weighing 65 to 80 g were used. Anesthesia was induced by inhalation of 2% halothane and maintained with 1% halothane in a 30% O_2/70% N_2O mix. Rectal and skull temperatures were monitored and kept at 37±0.5 °C using a heat lamp and ice pack. Under an operating microscope, a ventral neck incision was made and the bilateral common carotid arteries were gently exposed. Ischemia was induced by occluding the arteries with Sugita aneurysm clips. The clips were removed, restoration of blood flow through the arteries was confirmed visually, and the incision was sutured. The animal remained anesthetized and temperature was maintained for 50 min after ischemia.

The animals were divided into four groups: (1) sham-operated group ($n=5$): the bilateral common carotid arteries were exposed, no ischemia was given (sham-operation); (2) 2 min ischemia group ($n=5$): animals were subjected to a 2 min sublethal ischemic insult; (3) non-preconditioned group: 24 h after sham-operation a 5 min lethal ischemic insult was given; and (4) preconditioned group: the animals were pretreated with 2 min sublethal ischemia 24 h prior to 5 min lethal ischemia. Animals in groups 3 and 4 were given recovery times of 1 h, 6 h, 1 day, or 7 days (all subgroups $n=5$). The experimental protocol is summarized in Fig. 1. rCBF measurements were done 24 h after sham or 2 min ischemic treatment in groups 1 and 2 and at the designated time period in groups 3 and 4.

rCBF measurement

Quantitative [^{14}C] iodoantipyrine autoradiography technique was used to determine rCBF in the hippocampus (CA1 subfield, CA3 subfield, dentate gyrus) and other brain regions (frontal cortex, temporal cortex, caudate putamen, thalamus, hypothalamus, amygdala, substantia nigra, and mamillary body). For the autoradiographic determination of rCBF, the femoral artery and vein were cannulated for arterial blood sampling and radioisotope injection, respectively. At the designated time, 5.55 MBq/kg of [^{14}C] iodoantipyrine (American Radiolabeled Chemicals Inc. St. Louis, USA) dissolved in 0.5 ml physiological saline was injected intravenously for 30 s, and arterial blood samples were collected. The animals were then immediately sacrificed by decapitation. The brains were quickly removed and frozen (−60 °C) in isopentane chilled by liquid nitrogen. The brain was then sectioned (20 μm) coronally in a −20 °C cryostat. Slices were mounted on cover glasses, dried at 40 °C and exposed to X-ray film (Kodak), with [^{14}C] standards in cassettes, for 14 days. The radioactivity of the arterial blood samples was determined with a liquid scintillation counter. The film was developed, and the optical density of each brain was measured by means of a microcomputer imaging device (MCID) system (Imaging Research Inc.). The rCBF values were calculated as described by Sakurada et al. (1978) using a tissue–blood partition coefficient of 0.8. The rCBF values are expressed as means±S.D. (ml/100 g/min).

Physiological variables

Because removal of a large volume of blood may affect the systemic and cerebral circulations in these small animals, sepa-

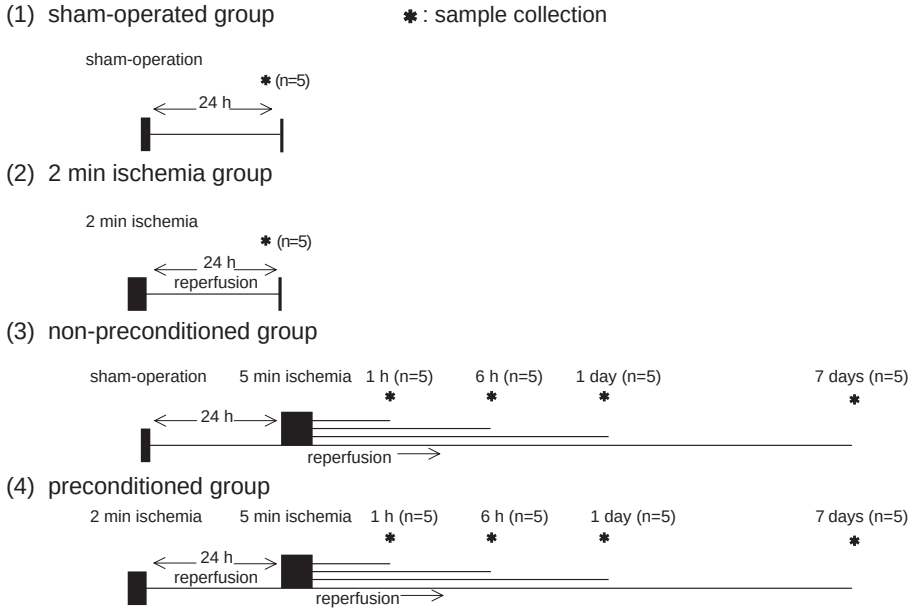

Fig. 1. Experimental protocol. Diagrams show the experimental protocol; n is the number of animals in each experimental group.