Progesterone treatment before experimental hypoxia-ischemia enhances the expression of glucose transporter proteins GLUT1 and GLUT3 in neonatal rats

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ABSTRACT

Progesterone is an efficient candidate for treating stroke and traumatic brain damage. The current study was designed to investigate the effects of progesterone on glucose transporter proteins (GLUT1 and GLUT3) during hypoxic-ischemic injury in a neonatal rat model. We demonstrated strong staining for GLUT1 in the walls of blood vessels and GLUT3 immunoreactivity in hippocampal neurons after hypoxia-ischemia. Hypoxia-ischemia elevated GLUT1 and GLUT3 at both the mRNA and protein levels in the hippocampus, and pre-treatment with progesterone (8 mg/kg) further enhanced their accumulation until 24 h after hypoxic-ischemic injury. These results showed that progesterone treatment induced the accumulation of both GLUT1 and GLUT3 transporters, and an energy-compensation mechanism may be involved in the neuroprotective effect of progesterone during hypoxic-ischemic injury after cerebral ischemic attacks.

Keywords: hypoxic-ischemic injury; progesterone; GLUT1; GLUT3; stroke

INTRODUCTION

Focal cerebral ischemia, currently one of the leading causes of death and injury worldwide, has attracted considerable attention. Cerebral ischemia causes blood flow reduction and leads to impaired oxygen and glucose delivery⁵. Lethal brain damage mainly results from neuron death, and the shortage of metabolic energy supply is one cause for the death of neurons. Furthermore, oxygen and glucose depletion impairs ion transport, induces the change of membrane potential and finally leads to the depolarization of neurons⁵⁶⁷. Therefore, as a primary energy substrate for mammalian brain metabolism, glucose is essential for the maintenance of neuronal function, especially in focal cerebral ischemia. The delivery of energy substrates such as glucose from blood to brain always requires facilitation by the glucose transporter (GLUT) proteins. Six isoforms of GLUT proteins are expressed in the mammalian brain, among which GLUT1 and GLUT3 are predominant. GLUT1 transports glucose across the endothelial cells of the blood-brain barrier (BBB), and then GLUT3 helps glucose to pass through the neuronal cell membrane. Limitations in the functions of GLUTs lead to abnormal brain function and neuron death⁵⁸.

In order to model human stroke, middle cerebral artery thread-occlusion in the rat is used in stroke pathophysiology and therapeutic research. Many reports have shown that alterations in the expression of GLUTs occur in cerebral ischemia⁵⁸⁷. And the evolution of brain damage from hypoxic ischemia in the neonatal rat involves major changes in GLUT1 and GLUT3 mRNA expression⁹. Induced expression of GLUT1 is detectable at 12 h to 7 days of recovery in the rat cerebral cortex after transient global ischemia⁹.
Moreover, reduction in GLUT1 mRNA and protein occur in glial cells and astroglia in animal models\[6, 9\]. Adult rats with middle cerebral arterial ischemia also show a progressive increase in brain GLUT3 concentration\[10\]. The reduction or increment in GLUT expression may serve as a molecular indicator of the stress of temporary reduction of blood flow.

A broad range of evidence shows that progesterone is neuroprotective under pathological conditions by modulating the BBB, interacting with the inflammatory cascade, decreasing the development of cerebral edema, limiting apoptosis, and protecting neurons distal to the injury that would normally die\[11-13\]. Clinically, progesterone treatment has neuroprotective benefits in several phase I and phase II clinical trials on traumatic brain injury and brain-injured children\[12, 14\]. A recent hypothesis suggests that the involvement of progesterone in protecting the fetus during development may recapitulate its effects in the treatment of traumatic and degenerative disorders of the brain\[12\].

The role of progesterone as a pleiotropic neurosteroid for brain injury has been well addressed. However, its regulatory effect on ischemic energy metabolism, especially on GLUT proteins, is still unrevealed. The current study aimed to investigate whether progesterone regulates the expression of GLUT proteins in the hippocampus after cerebral hypoxia-ischemia in infant rats.

MATERIALS AND METHODS

Animals

Timed pregnant Sprague-Dawley rats were housed individually in cages. Pups were housed with their dam after birth under a 12:12-h light-dark cycle in accordance with the guidelines and regulations of the Animal Care and Use Committee. Pups were randomized to the following groups: control, hypoxic-ischemic, sham-operated, and progesterone-pretreated hypoxic-ischemic (n = 12/group). Progesterone was dissolved in sesame oil and given as a single dose of 8 mg/kg by i.p. injection 30 min before the ischemia surgery. In the sham-operated group the same volume of sesame oil without progesterone was injected.

Induction of Hypoxia-Ischemia

Male and female 7-day-old rats were anesthetized by inhalation of 0.1% isoflurane in oxygen. The pups were kept at 37°C as the right common carotid artery was exposed and then ligated with surgical silk through a near-midline incision. After the wound was closed, all pups were allowed to recover in an incubator perfused with 8% oxygen-balanced nitrogen at 37°C for 2 h. In sham-operated rats, the right common carotid artery was exposed without ligation and they were not subjected to hypoxia. The operated pups were returned to the dams after hypoxic exposure. The animal study protocol was approved by the Institutional Animal Care and Use Committee.

Histological Examination

Pyramidal cell loss was assessed at 24 h after hypoxic-ischemic injury by hematoxylin and eosin staining. Pups were anesthetized with pentobarbital followed by transcardiac perfusion with 4% paraformaldehyde in PBS buffer. Brains were immersed in fixative and processed for paraffin embedding. Coronal sections (4 μm thick) were cut at the level of the hippocampus. Every sixth section was collected and stained with hematoxylin and eosin. Cells in the pyramidal layer of CA1 were counted under a light microscope at 400× magnification.

Immunohistochemistry Assay

Pups were killed under deep anesthesia and the brain was fixed in freshly-prepared paraformaldehyde (4%) for 40 min at 4°C. For immunohistochemical analysis, the tissue was cut into 16-μm sections on a freezing microtome. After heat-induced epitope retrieval and washing with TBS/Tween 20, sections were blocked with normal goat serum and then incubated with GLUT1 and GLUT3 rabbit polyclonal antibodies (1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Negative controls were sections without the primary antibody. After washing, sections were incubated with biotinylated secondary antibody for 1 h in a humidified chamber. Antibody reactions were detected with the streptavidin-biotin-peroxidase reaction following standard procedures (Zhongshan Golden Bridge Biotechnology Co., Ltd, Beijing, China). Positive cells per cm² were counted in 30 views in each group, based on stereology measurements.

Western Blot Analysis

Pups were killed under anesthesia at 24 h after hypoxic-ischemic injury. The hippocampus was frozen in liquid nitrogen and then kept at −80°C. The tissue was homogenized in ice-cold lysis buffer containing 0.2 mol/L TBS, 1 mmol/L