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Ischemia

Erythropoietin attenuates neuronal injury and potentiates the expression of pCREB in anterior horn after transient spinal cord ischemia in rats

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Abstract

Background: Recent studies have suggested that EPO activates the CREB transcription pathway and increases BDNF expression and production, which contributes to EPO-mediated neuroprotection. We investigated whether EPO has a neuroprotective effect against ISCI in rats and examined the involvement of CREB protein phosphorylation in this process.

Methods: Spinal cord ischemia was produced by balloon occlusion of the abdominal aorta below the branching point of the left subclavian artery for 5 minutes, and rHu-EPO (1000 U/kg BW) was administered intravenously after the onset of the reperfusion. Neurologic status was assessed at 1, 24, and, 48 hours. After the end of 48 hours, spinal cords were harvested for histopathologic analysis and immunohistochemistry for pCREB.

Results: All sham-operated rats had a normal neurologic outcome, whereas all ischemic rats suffered severe neurologic deficits after ISCI. Erythropoietin treatment was found to accelerate recovery of motor deficits and prevent the loss of motoneurons in the spinal cord after transient ischemia. Ischemic spinal cord injury induced the phosphorylation of pCREB at the anterior horn of the spinal cord, and EPO treatment significantly potentiated expression of pCREB increase at the anterior horn of the spinal cord.

Conclusions: These results demonstrate that a single dose of EPO given before ISCI provides significant neuroprotection and potentiates the expression of pCREB in this region.
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Keywords: Aorta; Spinal cord ischemia; Erythropoietin; pCREB; Neuroprotection; Rat

1. Introduction

Ischemic spinal cord injury remains the most dreaded complication of operations for treatment of descending thoracic and thoracoabdominal aortic aneurysms [11,13]. The necessity for aortic cross-clamping carries a risk of distal organ ischemia including kidneys, liver, intestines, and spinal cord. The incidence of spinal cord complications ranges from 8% to 30% and depends on the nature and extent of the disease and the duration of aortic cross-clamping [4]. Neuronal injury arising from spinal cord

Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; BW, body weight; cAMP, cyclic adenosine monophosphate; CREB, cyclic adenosine monophosphate responsive element binding protein; EPO, erythropoietin; ISCI, ischemic spinal cord injury; MAP, mitogen-activated protein; MSDI, motor sensory deficit index; NGF, nerve growth factor; PBS, phosphate-buffered saline; pCREB, phosphorylated CREB; pCREB-IR, pCREB immunoreactivity; rHu-EPO, recombinant human erythropoietin; VEGF, vascular endothelial growth factor.

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ischemia is believed to result from diverse but interrelated processes such as glutamate-mediated excitotoxicity, nitric oxide overproduction, inflammation, apoptosis, and free-radical generation [30]. These cellular stimuli are known to activate members of the MAP kinase family, which participate in numerous intracellular signaling pathways that can initiate reparative processes or cell death. Various agents and techniques that ameliorate these different processes have been tested in experimental model of spinal cord ischemia [11,13,25].

Erythropoietin is a hematopoietic cytokine hormone that has recently been shown to exert neuroprotection against various insults such as in vitro and in vivo. Erythropoietin and EPO receptors have both been reported in the brain cortex, cerebellum, hippocampus, pituitary gland, and spinal cord [24]. Erythropoietin has been reported to activate specific receptors in the central nervous system and was found to be neurotrophic and neuroprotective against to excitotoxicity, ischemia, hypoxia, trauma, and inflammation, and serum growth factor deprivation in both in vitro and in vivo models [18,24,40]. Acute and delayed beneficial action of systemically administered EPO has also been reported in rabbit ISCI [3].

The mechanisms of EPO to produce neuroprotective effects are reduction in glutamate toxicity, increased production of neuronal anti-apoptotic factors, reduced nitric oxide–mediated injury, anti-inflammatory effects, and antioxidant properties [18]. These findings have greatly enhanced the value of EPO as possible therapeutic strategy and focused attention on the molecular mechanisms underlying its neuroprotective effect.

CREB is a transcription factor with multiple functions [26,33] and is believed to play a key role in cell survival [2,7,39]. Reportedly, oxidant injury regulates the activity of CREB and thus survival [5,15,17,29]. CREB is constitutively expressed in neuronal nuclei, and its activation occurs via phosphorylation at serine 133 (Ser133) by various kinases, including MAP kinases [26,33]. Extracellular signal-regulated kinase phosphorylates CREB through p90rsk. Accordingly, various signaling cascades converge for CREB phosphorylation, such that CREB is now known to play a pivotal role in many physiologic activities including neuronal development, regeneration, memory function, synaptic plasticity, and cell repair [1,6,14,16,28,31,32,38]. For instance, CREB mediates neuronal responses to various neurotrophins including BDNF and NGF, which regulate neuronal survival, differentiation, neurogenesis, and synaptic function [8]. Conversely, CREB itself controls BDNF transcription [36]. CREB activation is an important event in neural plasticity and neuroprotection against injury in the developing brain, and drugs that activate cAMP-CREB signaling would provide novel therapeutic approaches for the treatment of hypoxic-ischemic brain injury [34,39]. Recently, it has been reported that EPO activates the CREB transcription pathway and increases BDNF expression and production, and this increase contributes to EPO-mediated neuroprotection in primary hippocampal neurons [37]. Another astonishing finding about the neuroprotective effect of EPO is that treatment of stroke with EPO enhances neurogenesis and angiogenesis, and improves neurologic function in rats by increasing VEGF and BDNF [40].

In this study, we investigated whether EPO has a neuroprotective effect against ISCI in rats and examined the possible involvement of CREB protein phosphorylation in this process.

2. Material and methods

2.1. Animal care

Seventeen Wistar albino rats (4-5 months old, male) weighing 300 to 375 g were used in this study. Animals were housed under standard conditions in the Animal Research Laboratory at Dokuz Eylül University. The study protocol was approved by the Animal Research Ethical Committee of Dokuz Eylül University.

2.2. Study groups

Animals were randomly divided into 3 groups.

1. Sham operation (n = 3): underwent the surgical procedure but the aorta was not occluded.
2. Control group (ischemia + saline) (n = 7): received normal saline intravenously immediately after the onset of the reperfusion.
3. EPO group (ischemia + EPO) (n = 7): received rHu-EPO (Eprex, Cilag, Zug, Switzerland) administered intravenously immediately after the onset of the reperfusion at a dose of 1000 U/kg BW.

2.3. Surgical procedure

Anesthesia was induced with 2% to 3% halothane in oxygen and with 1.5% halothane (in 100% O_2) delivered through a facemask during the surgery. The end-tidal carbon dioxide and halothane concentrations were monitored with a capnograph (Anesthesia Gas Monitoring 1304, Bruel and Kjaer, Naerum, Denmark) during surgery. The left femoral artery was also cannulated for monitoring the arterial blood pressure and arterial blood gases. Temperature was measured with a rectal probe and controlled by feedback to the heating lamp. The rats were kept at 37.0°C ± 0.5°C during the ischemic period.

Spinal cord ischemia was produced as described by Gilad and Gilad [12]. In brief, a balloon catheter was inserted (F2 Fogarty) through the abdominal aorta, below the kidneys, and the balloon (0.25 mL air) was inflated below the branching point of the left subclavian artery for 5 minutes, thereby blocking blood flow completely. Thereafter, the catheter was deflated and removed, and the aorta was closed, using a piece of the catheter as a bridging cannula. It was reported that longer periods of ischemia in this model may result in pulmonary edema and death within 24 hours after reperfusion. Heparin (200 U) was administrated as an intravenous bolus before aortic occlusion.

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