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Research report

The role of nitric oxide in the development of cortical spreading depression-induced tolerance to transient focal cerebral ischemia in rats

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Abstract

Cortical spreading depression (CSD) has been documented to confer ischemic tolerance on brain. Although nitric oxide (NO) is a crucial mediator in preconditioning under certain circumstances, the role of NO in CSD-induced neuroprotection is unclear. We examined the effect of L-NAME, an inhibitor of NO synthase, on CSD-induced tolerance against transient focal cerebral ischemia. A solution of 0.5 M KCl was applied for 2 h on the right hemisphere to induce CSD. Animals received either vehicle or L-NAME (4 mg/kg, iv) 30 min before CSD. Temporary occlusion (120 min) of the right middle cerebral artery was induced 4 days after preconditioning and the infarct volume was measured. Additionally, ERK 1/2 activation and cyclooxygenase-2 (COX-2) expression in the cerebral cortex were examined by Western blotting analysis immediately after cessation of CSD, or at 1, 2, 4, 8, and 24 h after CSD. CSD reduced infarct volume from 275 ± 15 mm^3 (mean ± SEM) in the non-CSD group to 155 ± 14 mm^3 in the CSD group ($P < 0.05$). L-NAME abolished this protection (281 ± 14 mm^3; $P < 0.05$ vs. CSD group). Elevated ERK activation and COX-2 expression were observed immediately after or 8 h after preconditioning, respectively. Those responses are significantly augmented by L-NAME (3-fold for ERK and 4-fold for COX-2). These results suggest a crucial role of NO in the establishment of preconditioning with CSD.

Theme: Diseases of the nervous system
Topic: Ischemia

Keywords: Cerebral blood flow; Brain ischemia; Ischemic preconditioning; Cerebral circulation; Cerebral ischemia, transient; Cyclooxygenase

1. Introduction

Cortical spreading depression (CSD) is characterized by a propagating depolarization wave that moves relatively slowly across the cortical surface [25]. Several studies have demonstrated that CSD elicited in normal brain induces strong and sustained tolerance to global [11,14] or focal cerebral ischemia [15,16,19,28]. However, the factors linking CSD with the development of ischemic tolerance are complex and controversial.

CSD has been reported to generate nitric oxide (NO), a key signaling molecule, at high levels in cortex [21]. Although several studies have revealed an obligatory role of the NO generating pathway in the development of brain preconditioning with hypoxia, oxygen glucose deprivation, or low dose lipopolysaccharide injection [5,6,20], no studies have examined whether NO participates in the development of CSD-induced ischemic tolerance.

The purpose of this study was to investigate the role of NO in the development of CSD-induced ischemic tolerance

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in rats. Specifically, we examined the effect of reduced NO production via administration of Nω-nitro-L-arginine methyl ester (L-NAME) on CSD-induced neuroprotection against transient, focal cerebral ischemia. Additionally, we also examined effects of L-NAME on activation of extracellular signal-regulated kinases (ERK) 1/2 and cyclooxygenase-2 (COX-2) protein expression following CSD. ERK 1/2 and COX-2 are thought to be early indicators of cellular stress [10,18] and have been reported to be involved in the development of ischemic preconditioning of brain and heart [1,10].

2. Materials and methods

2.1. Experimental design

We used a total 96 Male Wistar rats (250–320 g). All animal protocols were approved by the Institutional Animal Care and Use Committee.

Forty-eight rats were used to evaluate infarct volume due to transient right middle cerebral artery occlusion (MCAO) induced at 4 days after CSD. There were four subgroups studied. In the first group (Control group: n = 12), sham preconditioning (no CSD) was provided by topical application of saline to the cortical surface. In the second group (CSD group: n = 12), vehicle (saline) was injected intravenously 30 min before induction of CSD by topical application of 0.5 M KCl. In the third group (CSD with L-NAME group: n = 12), L-NAME (4 mg/kg) was injected instead of vehicle 30 min prior to the induction of CSD. In the fourth group (L-NAME alone group: n = 12), L-NAME (4 mg/kg, iv) was given 4 days before transient MCAO without preconditioning with CSD.

Another 48 rats were used for the investigation of ERK 1/2 activation and COX-2 expression following CSD alone (without MCAO) by Western blotting analysis. The rats were given L-NAME (4 mg/kg; n = 24) or saline (n = 24) intravenously at 30 min before induction of CSD.

2.2. CSD induction

After the induction of anesthesia with 5% halothane in oxygen, rats were ventilated with 1.0% halothane in a 70:30 gas mixture of N_2O and O_2. The femoral artery was cannulated to monitor mean blood pressure and to provide blood samples for glucose, pH, PCO_2, and PO_2. The femoral vein was also cannulated for the injection of L-NAME (Sigma) or saline 30 min before induction of CSD. Then the head of the animal was fixed in a stereotaxic frame. The skull was exposed by a longitudinal midline skin incision. Then three small burr holes were carefully made under a surgical microscope to prevent injury to the dura mater. The first one (1-mm diameter) was drilled over the right frontoparietal cortex (bregma −1.5 mm; lateral 2.5 mm) for placement of a Ag–AgCl electrode (1-mm diameter, World Precision Instruments) for monitoring of DC potentials. The second one (2 mm diameter) was also made over the right frontoparietal cortex (bregma −1.5 mm; lateral 5 mm) to position a laser Doppler flow probe (Multichannel Laser Doppler System, PERIMED) for measurement of cerebral blood flow (CBF). The third one (2-mm diameter) was drilled over the right occipital cortex (bregma −7 mm; lateral 5 mm) for the topical application of 0.5 M KCl to elicit CSD or of NaCl for sham preconditioning. In rats to be used for brain sampling for Western blotting analysis, three similarly placed burr holes were also made on the left side of the skull to exclude the influence of surgical invasion and infection on the cellular responses after preconditioning. After the fixation of a reference platinum electrode in the neck skin, the Ag–AgCl electrode was connected to a DC amplifier (DAM 50, Differential Amplifier, World Precision Instruments). Rectal temperature of rats was maintained at normal values (36.5–37.5 °C) throughout the experiments by a heating pad. The CSD on the right side hemisphere was induced by placing a cotton ball soaked with 0.5 M KCl into the burr hole. For the control group, a cotton ball soaked with NaCl instead of KCl was applied. For the rats used for Western blotting analysis, a cotton ball soaked with NaCl was also applied to the burr hole made over the left occipital cortex. The cotton ball was replaced every 15 min for a period of 2 h. To stop the induction of CSD, the burr hole was rinsed with saline three times. The burr holes were filled up with dental cement and after removing the catheter all wounds were closed. The rats were allowed to recover from anesthesia and returned to the cages. All recorded data of DC potential and CBF were continuously digitized and stored in the computer equipped with a multiple monitoring system (PeriSoft version 5.10, PERIMED) until analysis.

2.3. Transient focal cerebral ischemia

Transient MCAO was induced by the filament model as previously described [9]. Briefly, rats were ventilated spontaneously with 1.0% halothane in a 70:30 gas mixture of N_2O and O_2. The tail artery was cannulated to monitor mean blood pressure, blood glucose, and blood pH and gases. During surgery, the rectal temperature was maintained in a range between 36.5 °C and 37.5 °C by a heating pad. The right external and internal carotid arteries (ECA and ICA) were exposed through a midline neck incision. The ECA was cut between double ligations at the distal portion. A 4–0 monofilament nylon suture (Ethicon), its tip coated with silicon (Rhodoia RTV 1556 A and B), was introduced into the ICA via the ECA stump and advanced in the ICA approximately 20 mm from the carotid bifurcation. After the obstruction of the common carotid artery by a microvascular clip, the suture around the ECA stump was tightened and the incision was closed. Then anesthesia was discontinued and rats were kept in the cage during 120 min