Effect of Magnesium on Nitric Oxide Synthase of Neurons in Cortex during Early Period of Cerebral Ischemia

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Summary: To investigate the effect of magnesium on nitric oxide synthase (NOS) of neurons in cortex during early cerebral ischemic period, a rat model of middle cerebral artery occlusion (MCAO) was established. The results showed that the NOS activity of neurons in cortex was increased significantly at 15 min after MCAO, reached its peak at 30 min after MCAO and returned to normal levels at 60 min after MCAO. The NOS activity of neurons in the magnesium-treated group was decreased significantly as compared with that in the ischemic group at 15 min and 30 min after MCAO respectively. The results suggested that magnesium could inhibit the elevated NOS activity of neurons in cortex induced by cerebral ischemia.

Key words: cerebral ischemia; nitric oxide synthase; magnesium

Lots of animal experiments and clinical trials suggest that magnesium can protect against ischemic brain damage. Magnesium is a natural blocker of calcium and N-methyl-D-aspartate (NMDA) receptor. The neuroprotective mechanism of magnesium may lie in blocking overflux of calcium, inhibiting release of excitatory amino acid (EAA) and decreasing energy metabolism.

Recently, nitric oxide (NO) has been found to be involved in ischemic brain injury. During the early period of cerebral ischemia, the calcium-dependent nitric oxide synthase (NOS) activity in neurons is increased, which leads to overproduction of NO and results in neurotoxicity. The purpose of the present study was to investigate the effect of magnesium on the activity of NOS in cortical neurons during early cerebral ischemic period, so as to study the neuroprotective mechanism of magnesium.

1 MATERIALS AND METHODS

1.1 Animal Model

Forty-five adult male Wistar rats weighing 200–250 g were used in this experiment. Focal cerebral ischemia was induced as previously described by Tamura et al.[1]. Animals were anesthetized with chloral hydrate (400 mg/kg, i. p.). Body temperature was maintained at 35–36°C with a blanket and a lamp. After removal of the coronoid process of the mandible, a craniectomy was made, the dura was opened and the MCA was then occluded just after the point where it crossed the olfactory nerve.

1.2 Animal Groups

The rats were randomly assigned to the following groups: (1) sham-operated groups, in which rats were subjected to MCA occlusion and were sacrificed at various time points after onset of MCA occlusion (15 min, 30 min, 60 min, 180 min). (2) Magnesium-treated groups, in which rats were intravenously administered magnesium sulfate (500 mg/kg) at a velocity of 1.7 mg/min 3 h before the onset of ischemia. They were also sacrificed at various time points after onset of MCA occlusion (15 min, 30 min, 60 min, 180 min). Each subgroup included 5 rats.

1.3 NOS Assay: NADPH-diaphorase Histochemistry

NOS was assayed by the method described by Vincent et al[2]. The brains were rapidly removed and frozen on dry ice. Coronal brain sections (25 μm thick) were rinsed in phosphate buffer (0.1 mol/L) containing 0.2% Triton X-100 for 5 min, and incubated in 0.1 mmol/L PBS containing 0.2 mmol/L NBT, 1 mmol/L NADPH and 0.2% Triton X-100 at 37°C for 90 min. The characteristics of NOS positive neurons in ischemic cortex were observed. The parameters of the number of NOS positive neurons (neurons/mm²) and the optical density (OD) in ischemic cortex were measured by using MPAAS-500.

1.4 Statistical Analysis

All values were expressed as x ± s. Statistical analysis of values was performed using a one-way ANOVA.

2 RESULTS

2.1 Characteristics of NOS Positive Neurons

According to the densities of blue reactive products in neurons, the NADPH-diaphorase staining neurons, i.e. NOS positive neurons can be separated into 3 categories. (1) Weak positive: plasma faint blue, nucleus unstained. (2) Moderate positive: plasma, axons and dendrites blue, nucleus faint blue. (3) Intense positive: plasma, axons, dendrites and nucleus dark blue.

2.2 The Change of the Activity of NOS in Each Group

In the sham-operated group, there was only...
small amount of weakly to moderately stained neurons in the cerebral cortex (fig. 1, 4). At 15 min after ischemia, the neurons were stained moderately and the number of NOS positive neurons increased. At 30 min after ischemia, the intensely stained neurons were found and the number of NOS positive neurons increased to its peak (fig. 2, 5). At 60 min after ischemia, the staining and the number of NOS positive neurons returned to normal levels. As to magnesium-treated groups, at 15 min after ischemia, the neurons were stained weakly to moderately and the number of the NOS positive neurons increased; at 30 min after ischemia, the neurons were stained moderately to intensely, and the number of NOS positive neurons increased obviously (fig. 3, 6). The staining and number of the NOS positive neurons of magnesium-treated groups at 15, 30 min after ischemia was decreased significantly in comparison with that of ischemic groups respectively. At 60, 180 min after ischemia, there was no significant differences between ischemic and magnesium-treated groups. The changes observed under a microscope were confirmed by the image analysis (table 1, 2).

Fig. 1 Weak positive NOS neurons in cortex of sham-operated rats (X 40)
Fig. 2 Intense positive NOS neurons in cortex of rats exposed to MCAO for 30 min (X 40)

Fig. 3 Moderate positive NOS neurons in cortex of rats treated with magnesium and exposed to MCAO for 30 min (X 40)
Fig. 4 Small amount of NOS positive neurons scattered in cortex of sham-operated rats (X 40)