Effect of Electroacupuncture on TRPM7 mRNA Expression after Cerebral Ischemia/reperfusion in Rats via TrkA Pathway*

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Summary: The effect of electroacupuncture (EA) on TRPM7 mRNA expression of focal cerebral ischemia in rats and further the role of EA in the relationship between TRPM7 and trkA pathway was investigated. Thirty SD rats were randomly divided into 5 groups: normal group, ischemia/reperfusion group, EA treated group (ischemic rats with EA treatment), TE infusion group (ischemic rats with EA treatment and TE buffer infusion), AS-ODN group (ischemic rats with EA treatment and antisense trkA oligonucleotide infusion). The stroke animal model was established by the modified method of middle cerebral artery occlusion. Antisense trkA oligonucleotide that blocked NGF's effects was injected into cerebroventricle before EA. The TRPM7 mRNA was detected by RT-PCR method. The results showed that there were low TRPM7 mRNA levels in cortex and hippocampus in normal group. Compared with normal group, TRPM7 mRNA expression was increased significantly in ischemia/reperfusion group ($P<0.05$). A significant reduction in the expression of TRPM7 mRNA was found in EA treated group in contrast to ischemia/reperfusion group ($P<0.05$). The expression of TRPM7 mRNA in AS-ODN group was remarkably increased compared with EA treated group and TE infusion group ($P<0.05$). The results indicated that TRPM7 channels in the ischemic cortex and hippocampus in rats might play a key role in ischemic brain injury. EA could reverse the overexpression of TRPM7 in cerebral ischemia/reperfusion rats. And the inhibitory effect of EA on TRPM7 channels might be through trkA pathway.
Key words: TRPM7; ischemia/reperfusion; electroacupuncture; antisense trkA oligonucleotide

TRPM7 ion channel protein is a member of the transient receptor potential (TRP) cation channel superfamily. It is an unusual bifunctional protein that contains an α-kinase domain fused to a Ca^{2+}-permeable cation channel. Aarts and colleagues provide evidence that TRPM7 initiates Ca^{2+} overload. TRPM7 may play a key role in anoxic neuronal death[1]. Electroacupuncture (EA) has been shown to be an effective treatment on stroke. The detailed mechanisms mediating the beneficial effects of EA on stroke are still unknown. Elucidated by recent studies, EA treatment acts as the role of neuroprotection by suppressing neuronal apoptosis[2]. In our previous study, after cerebral ischemia EA can induce the expression of nerve growth factor receptor, tropomyosin-related kinase A (trkA), which mediates neuroprotective effects[3]. However, little evidence is available on the effect of EA and trkA pathway on the expression of TRPM7. In the present study, we investigated the effect of EA on TRPM7 mRNA expression of focal cerebral ischemia in rats and further the role of EA in the relationship between TRPM7 and trkA pathway.

1 MATERIALS AND METHODS

1.1 Animal Grouping and Models

Thirty healthy Sprague Dawley (SD) rats of both sexes, weighing 200 to 250 g were used (Center of Experimental Animal, Tongji Medical College). The rats were randomly divided into 5 groups ($n=6$ in each group): normal group, ischemia/reperfusion group, EA treated group (ischemic rats with EA treatment), TE infusion group (ischemic rats with EA treatment and TE buffer infusion), AS-ODN group (ischemic rats with EA treatment and antisense trkA oligonucleotide infusion). The surgical procedure was described previously with slight modification[4]. Briefly, the rats were first anesthetized by 10 % chloral hydrate (350 mg/kg). Then the right common carotid artery was carefully exposed, and the right internal and external carotid arteries were dissected out. A 60-mm-long 4−0 nylon surgical thread was inserted from the right carotid bifurcation. The thread was passed through the internal carotid artery to occlude the middle cerebral artery (MCA). Approximately 18 mm of nylon surgical thread was inserted into the right internal carotid artery. After a 30-min occlusion of the right MCA, the nylon surgical thread was removed to allow reperfusion of the ischemic area. Finally, the animals were decapitated 24 h after reperfusion.

1.2 Intracerebroventricular Injection

Antisense oligonucleotides directed to trkA have been manufactured by Shanghai Sangon Biological Engineering Technology and Service Co., Ltd (China). The antisense trkA sequence (5′-AACTGTTGTTGTGTCC-3′ [5]) corresponds to nucleotides 623−638 of rat trkA, fully phosphorothioated. We dissolved the oligonucleotides in TE buffer containing 10 mmol/L Tris-Cl, pH 7.2, and 1 mmol/L EDTA to a final con-

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centration of 1000 μmol/L. We infused 5 μl of antisense trkA oligonucleotide and vehicle (TE buffer) respectively with a Hamilton syringe with a 27-gauge needle before EA. The location of each injection was 2 mm rostral, 1.5 mm later to bregma, and 2 mm deep to the skull surface.

1.3 EA Treatment

EA was applied at the anatomical points of Renzhong (DU26) and Chengjiang (RN24) 30 min before and after cerebral ischemia. The wave was 4—16 Hz. The initial intensity of the stimulation was 1 V, increased 1 V per 10 min, and the terminal intensity was 3 V. EA treatment lasted 30 min.

1.4 Reverse Transcriptase (RT)-PCR

Brain tissues from the cortex and hippocampus of the ischemic side were dissected. Tissue samples (50—100 mg) were lysed by 1 ml Trizol solution (Omega Bio-tech Inc., USA) to extract total RNA.

A standardized semiquantitative PCR method was used based on amplification of the target gene, TRPM7, and a constitutively expressed gene, β-actin. Total RNA (2 μg) was reverse transcribed, then aliquots of cDNA (5 μl) were amplified by following the Kit instructions (Fermentas, USA). Forward primer of TRPM7 (Chak, GenBank NM_053705): 5′-CTGAAGAGGAATGACTACAC-3′ (1211—1230); reverse primer of TRPM7: 5′-ACAGGGAAAAAGAGAGGGAG-3′ (1851—1870)[6]. Forward primer of β-actin (GenBank NM_031144): 5′-CATCCAGGCTGTGTTGTCCC-3′ (486 — 505); reverse primer of β-actin: 5′-TTCTCTTTAATGTCACGCACG-3′ (705—725). Amplification reactions were overlaid with light mineral oil and held at 95 ℃ for "hot-start" PCR for 3 min, then run in an automated thermal cycle for 30 cycles. Each cycle consisted of 95 ℃ for 1 min, 55 ℃ for 40 s, and 72 ℃ for 1 min, with a final extension for 10 min at 72 ℃. Sample electrophoreses were photographed with a Kodak Digital Science Scanner and analysis was carried out using the same system. Results were expressed as relative intensity in arbitrary units compared to the control value.

1.5 Statistical Analysis

Data were expressed as $\bar{x} \pm s$. Statistical comparisons were made using analysis of variance (ANOVA). In any cases, it was considered to be statistically significant when $P<0.05$.

2 RESULTS

2.1 The Expression of TRPM7 mRNA after Focal Cerebral Ischemia/Reperfusion

The expression of TRPM7 mRNA in cortex (C) and hippocampus (H) was detected by RT-PCR in the 5 groups. The results showed that there were low TRPM7 mRNA levels in cortex and hippocampus in normal group (C: 1.2340 ± 0.10900, H: 1.2420 ± 1.12657, fig. 1). Compared with normal group, TRPM7 mRNA expression in ischemia/reperfusion group was increased significantly at 24 h after reperfusion in cortex and hippocampus (C: 1.7183 ± 0.26011 vs 1.2340 ± 0.10900, H: 1.6967 ± 0.27340 vs 1.2420 ± 1.12657, $P<0.05$, fig. 1).

2.2 EA Treatment Reversed the Overexpression of TRPM7 mRNA after Focal Cerebral Ischemia/Reperfusion

EA was applied 30 min before and after MCA occlusion in EA treated group. As shown in fig. 2, the expression of TRPM7 mRNA was significantly decreased in EA treated group as compared with ischemia/reperfusion group in hippocampus and cortex of cerebral ischemic rats (C: 1.1417 ± 0.17244 vs 1.7183 ± 0.26011, H: 1.0967 ± 0.10633 vs 1.6967 ± 0.27340, $P<0.05$, fig. 2). The result indicated that EA could suppress the overexpression of TRPM7 mRNA after focal cerebral ischemia/reperfusion.

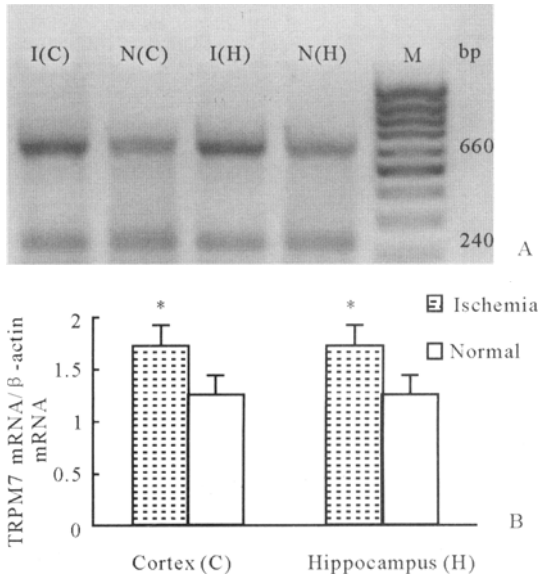

Fig. 1 Expression of TRPM7 in hippocampus and cortex of cerebral ischemic rats

A: M: Marker; N (H): Hippocampal sample in normal group; I (H): Hippocampal sample in ischemia/reperfusion group; N (C): Cortical sample in normal group; I (C): Cortical sample in ischemia/reperfusion group. The upper bands: TRPM7 RT-PCR products; the lower bands: β-actin RT-PCR products;

B: The expression of TRPM7 mRNA in ischemia/reperfusion group was increased significantly as compared with normal group (* $P<0.05$)

2.3 Antisense trkA Oligonucleotide Inhibited the Effect of EA on TRPM7

To determine whether the expression of TRPM7 was mediated by trkA pathway, antisense trkA oligonucleotide that blocked NGFs effects was injected into cerebroventricle before EA in AS-ODN group. The expression of TRPM7 mRNA in AS-ODN group (C: 1.4333 ± 0.15095, H: 1.3933 ± 0.12160) was increased remarkably in contrast to EA treated group and TE infusion group (C: 1.0017 ± 0.07111, H: 1.0100 ± 0.11679, $P<0.05$, fig. 3). At the same time, there was no significant difference in the expression be-

tween EA treated group and TE infusion group ($P>0.05$, fig. 3). The data demonstrated that the effect of EA on TRPM7 was inhibited by antisense trkA oligonucleotide.

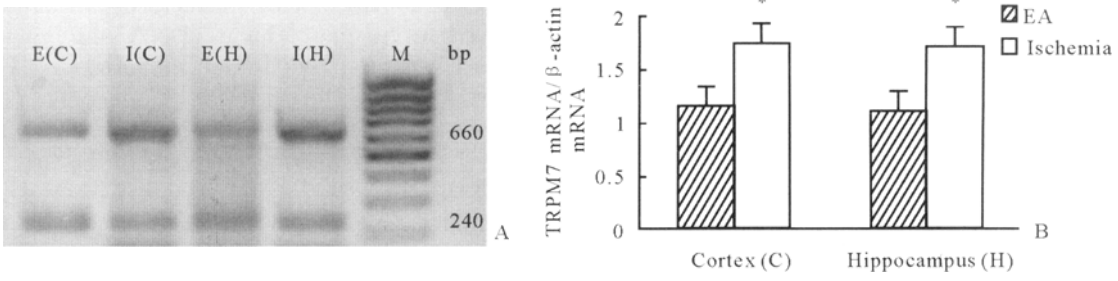

Fig. 2 Effect of EA treatment on TRPM7 mRNA levels in hippocampus and cortex of cerebral ischemic rats
A: M: marker; I (H): Hippocampal sample in ischemia/reperfusion group; E (H): Hippocampal sample in EA treated group; I (C): Cortical sample in ischemia/reperfusion group; E (C): Cortical sample in EA treated group. The upper bands: TRPM7 RT-PCR products ; the lower bands: β-actin RT-PCR products; B: The expression of TRPM7 mRNA in EA treated group was decreased significantly as compared with ischemia/reperfusion group ($^{*}P<0.05$)

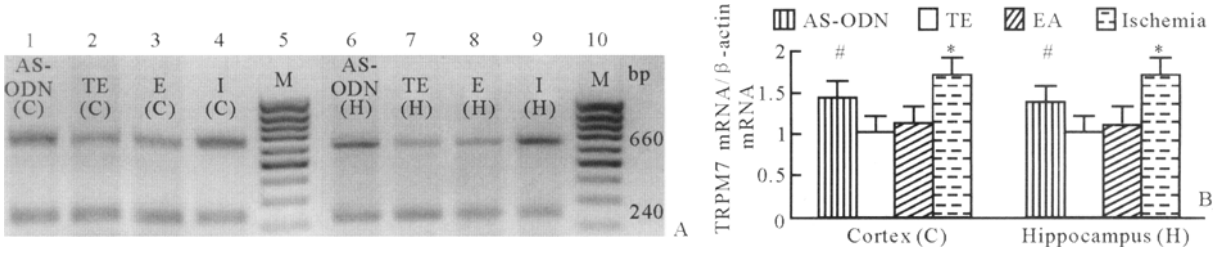

Fig. 3 Effect of trkA pathway on TRPM7 mRNA expression following EA in hippocampus and cortex of cerebral ischemic rats
A:1: Cortical sample in AS-ODN group; 2: Cortical sample in TE infusion group; 3: Cortical sample in EA treated group;
4: Cortical sample in ischemia/reperfusion group; 5 and 10: Marker;
6: Hippocampal sample in AS-ODN group; 7: Hippocampal sample in TE infusion group; 8: Hippocampal sample in EA treated group; 9: Hippocampal sample in ischemia/reperfusion group; The upper bands: TRPM7 RT-PCR products; the lower bands: β-actin RT-PCR products;
B: The expression of TRPM7 mRNA in AS-ODN group was increased remarkably as compared with EA treated group and TE infusion group ($^{\#}P<0.05$). The levels of TRPM7 mRNA in ischemia/reperfusion group were higher than those in the other groups ($^{*}P<0.05$)

3 DISCUSSION

Cerebral ischemia is a familiar disease in clinical practice. After acute MCA occlusion, the focal deprivation of oxygen and glucose in the ischemic core leads neurons necrosis quickly. But in the penumbra, the neurons undergo global inhibition of protein synthesis, disturbance of energy metabolism, prolonged depolarization, release of excitatory amino acid, free radical production and intracellular Ca^{2+} overload, and finally exhibit the morphological characteristic of apoptosis. The penumbra clearly has a limited life span and appears to undergo irreversible injury within a few hours unless neuroprotective therapy administered.

The term excitotoxicity describes the pathological consequences of the overstimulation of glutamate receptors, which occurs in brain ischemia/anoxia. It appears that excitotoxic glutamate receptor activity triggers the damaging processes, of which Ca^{2+} influx through N-methyl-D-aspartate glutamate receptors (NMDARs) is key. Previous studies have shown that excitotoxicity of glutamic acid after cerebral ischemia is the medium of NMDAR1 mRNA overexpression[7, 8]. It has been proved that when neurons are exposed to prolonged oxygen glucose deprivation (OGD), excitotoxic neuronal death can be eliminated by treating these cells with MK-801 (antagonist of NMDA). But antiexcitotoxic therapy (AET) has failed to prevent neuronal death if OGD is extended to 1.5 h or more and clinical trials of AET have generally failed to benefit patients[1]. This fact suggests that lethal signals in brain ischemia are not exclusively mediated through the NMDA-R associated channel. Aarts and colleagues unveiled a new lethal pathway. Their studies showed that even if NMDAR activity was permitted in the absence of MK-801 during OGD, depletion of TRPM7 by sRNAi could inhibit neuronal death. The neurons could survive over 3 h. So Aarts suspected that ex-

citotoxicity may become less pronounced with increasing durations of anoxia and TRPM7 channel, which allows Ca^{2+} entry into the neuron, may be the dominant pathway[1].

TRPM7 ion channel protein is a member of the TRP cation channel superfamily. Unique among ion channels, TRPM7 not only is a calcium-permeable non-selective ion channel but also has a functional C-terminal kinase. Aarts and colleagues suspected that TRPM7 channels could play a central role in anoxic neuronal death. On this basis, we investigated TRPM7 mRNA expression of focal cerebral ischemia in vivo. The data demonstrated that the expression of TRPM7 mRNA in the ischemic cortex and hippocampus was increased significantly at 24 h after reperfusion as compared with normal corresponding regions. It is known that TRPM7 allows inflow of massive amounts of Ca^{2+} in neurons and Ca^{2+} overload can trigger several downstream lethal reactions, which culminate in cell death. So we suggested that TRPM7 could also contribute to ischemic brain damage.

EA is a physical treatment in Chinese Traditional Medicine. Clinically, EA has been shown to produce beneficial effects on stroke patients. The detailed mechanisms, however, mediating the beneficial effects of EA on stroke are still unknown so far. The study of its mechanism can discover the secret of Chinese Traditional Medicine and supply directions of clinical work. In the present study, using a rat model of cerebral ischemia/reperfusion injury, we investigated the effect of EA on the expression of TRPM7. In contrast to ischemia/reperfusion group, TRPM7 mRNA expression in ischemic cortex and hippocampus was decreased significantly following EA. The result demonstrated the neurons could be protected by EA treatment, which might inhibit Ca^{2+} entry route through TRPM7 channels.

Nerve growth factor (NGF) is a member of neurotrophin superfamily that has been shown to protect neurons[9]. Many studies in neurons reveal major signaling pathways that may mediate survival by NGF through the high affinity neurotrophin receptor, trkA[10]. In our previous study, EA could induce the expression of trkA and raise element of inner suppression apoptosis after cerebral ischemia[3]. In the present study, we found that EA treatment withdrew the increase of the TRPM7 expression induced by ischemia. Interestingly, TRPM7 and trkA are both involved in the processes of transient cerebral ischemia, but little evidence is available on the effect of trkA pathway on the expression of TRPM7 channel. In the present study, we blocked NGF s effects by infusing antisense trkA phosphorothioate DNA oligonucleotide before EA. Then we found the effect of EA on TRPM7 was inhibited. The result demonstrated that trkA pathway could negatively regulate the TRPM7 channels during ischemia. The up-regulation of the trkA expression after EA treatment may be, in part, responsible for the decrease of induced high levels of TRPM7 after cerebral ischemia. These findings will be benefit for the clinical application of EA.

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