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Electroacupuncture pretreatment attenuates brain injury in a mouse model of cardiac arrest and cardiopulmonary resuscitation via the AKT/eNOS pathway

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ABSTRACT

Aims: This study aims to examine the effects of electroacupuncture (EA) pretreatment on brain injury after cardiac arrest and cardiopulmonary resuscitation (CA/CPR) and its underlying mechanisms.

Materials and methods: Adult male C57BL/6 mice were subjected to 6 min of cardiac arrest induced with a potassium chloride infusion and resuscitated by chest compressions and an epinephrine infusion. During the 3 days prior to CA/CRP, mice received EA pretreatment (1 mA, 2 Hz; daily session of 30 min) at the *Baihui* acupoint (GV20) once daily. Stimulation at a nonacupoint served as a control. In mechanistic studies, mice received the AKT inhibitor LY294002 or endothelial nitric oxide synthase (eNOS) inhibitor L-NIO 30 min before EA pretreatment. A neurological assessment was conducted 24 h after CA/CRP, followed by animal sacrifice and evaluation of physiological brain damage.

Key findings: CA/CPR resulted in severe brain injury as evidenced by neurological deficits and increased neuronal apoptosis, oxidative stress and the proinflammatory cytokines TNF-α and IL-6. EA pretreatment at the GV20 acupoint but not at a nonacupoint attenuated the neurological deficits and the pathological changes induced by CA/CPR. LY294002 or L-NIO eliminated the neuroprotective effects of the EA pretreatment.

Significance: This study showed that EA pretreatment at the GV20 acupoint can protect the brain from damage associated with globalized ischemia followed by reperfusion and that these protective effects occur via the AKT/eNOS signaling pathway.

1. Introduction

Cardiac arrest in the operating room and procedural areas is a very serious perioperative complication, which has a different spectrum of causes (e.g., hypovolemia, gas embolism, hyperkalemia, local anesthetic systemic toxicity, hypoxemia, and anaphylactic reactions) [1]. Cardiac arrest is associated with mortality rates as high as 58.4%, even with the lowest survivorship (< 20%) among patients who are elderly, exhibit a higher American Society of Anesthesiologists status, and undergo emergency procedures [2]. Brain injury after cardiac arrest/cardiopulmonary resuscitation (CA/CPR) is one of the important causes of death (23%) [3]. Even survivors after cardiac arrest showed severe brain injury manifested as coma, seizures, myoclonus, varying degrees of neurocognitive dysfunction, and brain death. Given that surgery is predictable, effective pretreatment to protect against brain injury after perioperative cardiac arrest is necessary and meaningful.

Strategies for neuroprotection include therapeutic hypothermia, calcium-sodium channel antagonists, *N*-methyl-D-aspartate receptor

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antagonists, growth factors, and protease inhibitors. Therapeutic hypothermia is currently the recommended form of intervention [3,4] but only offers 30% survival benefits to patients with successful cardiopulmonary resuscitation after cardiac arrest [5,6]. Other more effective treatments and approaches are still needed.

A growing number of studies have suggested the potential of electroacupuncture (EA) pretreatment as an effective neuroprotector. In rat studies, EA pretreatment protected against focal cerebral ischemia injury induced by middle cerebral artery occlusion, and it promoted neurological recovery by activating astrocytes or through regulation of the endocannabinoid system [7–10]. In an animal model of acute myocardial ischemia-reperfusion injury, EA treatment protected the brain by balancing the autonomic nervous system, suppressing neuronal cell apoptosis, blocking the activation of microglia cells, reducing oxidative stress and inhibiting systemic inflammation [11]. Whether and how EA pretreatment can protect against brain injury after CA/CPR remain unclear.

Endothelial nitric oxide synthase (eNOS) and its product nitric oxide (NO) play important roles in neuroprotection. Activation of PI3K/AKT/eNOS signaling mediated protection in rat cerebral ischemic injury, whereas eNOS deficiency enhanced the inflammatory response and cell death caused by CA/CPR in the heart, liver and brain of mice [12]. Mice given NO in breathing air for 23 h after CA/CPR showed a higher 10-day survival rate compared with rats not administered NO [13]. Administration of sodium sulfide, which produces nitrite that can be converted to NO, attenuated oxidative stress and neurological dysfunction caused by CA/CPR, and its neuroprotective effects were mediated via promotion of phosphorylation of AKT and eNOS in the brain and abolished in eNOS-deficient mice [14]. NO resulting from therapeutic nitrite modulated oxidative stress and limited neurological impairment after resuscitation following cardiac arrest [15,16].

Based on the literature, we hypothesize that EA pretreatment may exert neuroprotective effects against brain injury induced by CA/CPR through the AKT/eNOS signaling pathway. Therefore, we modified the CA/CPR mouse model to more closely mimic the human situation and explored the potential neuroprotective effects of EA pretreatment as well as the underlying mechanism.

2. Materials and methods

2.1. Ethics statement

All experiments were approved by the Institutional Animal Care and Use Committee of Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine and were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

2.2. Animal model of CA/CPR

Male C57BL/6 mice (8–12 weeks old, 23–25 g) were purchased from Shanghai Xipuer-Bikai Experimental Animal Company (Shanghai, China). The CA/CPR mouse model was prepared as previously described [17,18] with modifications. Mice were anesthetized with 3% isoflurane and underwent tracheal intubation. Cardiac arrest was induced by injecting potassium chloride (0.08 mg/g of body weight) in the femoral vein and confirmed by appearance of asystole on the electrocardiogram. During the procedure, head and body temperature was maintained at 37 ± 0.5 °C using a heating lamp. Chest compression and mechanical ventilation (FiO_2 = 1.0) were initiated 6 min after induction of cardiac arrest. Chest compression was performed using finger pressing at a frequency of ~300 bpm. A slow intravenous injection of 0.2–0.3 mL of epinephrine (16 μg epinephrine/mL, 0.9% saline) was started 30 s before CPR and stopped until mean arterial pressure remained stable at > 40 mmHg for 1 min, which was defined as return of spontaneous circulation. If spontaneous circulation did not return within 2.5 min, CPR was abandoned, and the mouse was euthanized. After 1-h recovery with mechanical ventilation, mice were returned to their cages and housed in a room maintained at 25 °C. Sham-operated animals were treated the same as the CA/CPR group, except they were given the saline injection instead of the potassium chloride injection.

Mice were euthanized 24 h after the procedure. Serum was collected, and hippocampal tissues were harvested for experiments.

2.3. EA pretreatment

EA pretreatment was performed using Han's Acupoint Nerve Stimulator (model LH202H, Beijing Huawei Zhongyi Technology). Mice were fixed in a prone position using a stereotaxic device. Electrodes were placed on the *Baihui* acupoint (GV20), and mice were given EA pretreatment (1 mA, 2 Hz) for 30 min once daily for three days before CA/CPR. Control-stimulated animals received EA pretreatment through electrodes placed 1 cm distal to the mouse tail base at a nonacupoint (NA-EA).

For inhibitor experiments, 0.6 mg/kg of AKT inhibitor (LY294002; Sigma Aldrich, USA) and 0.5 mg/kg of eNOS inhibitor (L-NIO; Sigma Aldrich, USA) were administered by intraperitoneal injection 30 min before EA pretreatment.

2.4. Neurological function assessment

Neurological function was assessed 24 h after CA/CPR or sham operation. The following five aspects [14] were assessed by an observer blinded to the treatment group: level of consciousness, corneal reflex, respirations, coordination and movement (Supplementary Table 1).

2.5. Serum hydrogen peroxide and NO concentration

Serum concentration levels of hydrogen peroxide were measured using a hydrogen peroxide assay kit (A064-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China), and NO was measured using a nitric oxide assay kit (A013-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) per the manufacturer's instructions [19].

2.6. Real-time quantitative polymerase chain reaction (qRT-PCR)

Total RNA was isolated from hippocampal tissue with Trizol (Invitrogen, USA). An aliquot (1 ng) was reverse-transcribed into cDNA using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher

Table 1
Primer sequences for qRT-PCR.

Primer	Sequence (5′-3′)	Transcript length	Transcript accession number
NOX2	Forward: GGTGATGTTAGTGGGAGC Reverse: AGGAAGTTGGCATTGTTC	213	FJ168469.1
NOX4	Forward: AAGATTTGCCTGGAAGAAC Reverse: TCAGAGGGATGATTGATGAC	159	NM_001285833.1
GAPDH	Forward: CTGCCCAGAACATCATCC Reverse: CTCAGATGCCTGCTTCAC	197	NM_001289726.1

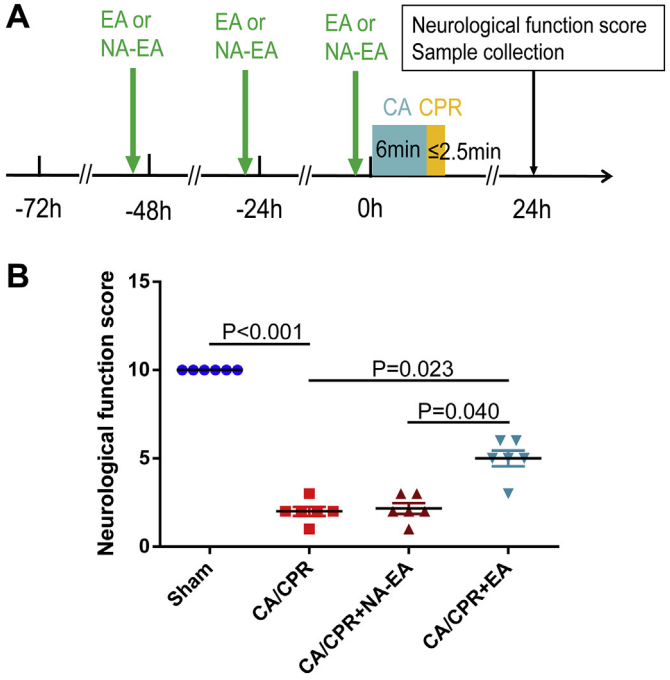

Fig. 1. EA pretreatment at the GV20 acupoint improves the neurological function score after CA/CPR. Mice underwent sham operation or the CA/CRP procedure with or without EA pretreatment at the GV20 acupoint or a non-acupoint. (A) Schematic of experimental timeline. (B) Mean neurological function score of mice at 24 h after the procedure. Data are mean ± SD ($n = 6$ mice per group). Abbreviations: CA, cardiac arrest; CPR, cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at GV20 acupoint; NA-EA, electroacupuncture pretreatment at nonacupoint.

Scientific, Waltham, MA, USA), which served as template in qRT-PCR reactions containing Maxima SYBR Green/ROX qPCR Master Mix (2×, Thermo Fisher Scientific) and appropriate primers (Table 1) run on an ABI-7300 thermal cycler (Applied Biosystems, CA). Levels of mRNAs encoding NADPH oxidase 2 (NOX2) and NADPH oxidase 4 (NOX4) were determined relative to levels of mRNA encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Relative expression level was calculated using the $2^{-\Delta\Delta Ct}$ method.

2.7. Western blotting

Total protein was extracted from the hippocampal samples, and protein concentration was estimated using the BCA protein assay kit (Nanjing Jiancheng Bioengineering Institute). The protein was separated by electrophoresis on either an 8% or 10% polyacrylamide gel (JRDUN Biotechnology, Shanghai, China) and then transferred onto a polyvinylidene fluoride membrane. Membranes were blocked with 5% nonfat dry milk or bovine serum albumin (BSA) for 1 h at room temperature. Primary antibodies were incubated at 4 °C overnight. Primary antibodies were purchased from Cell Signaling Technology (Danvers, MA) against the following proteins: AKT (#4685, 1:1000), p-AKT (Ser473) (#4060, 1:2000) and GAPDH (#5174, 1:2000). Primary antibodies were purchased from Abcam (Cambridge, UK) against the following proteins: NOX2 (ab80508, 1:1000), NOX4 (ab195524, 1:1000), eNOS (ab76198, 1:500) and p-eNOS (Ser1177) (ab215717, 1:1000). Membranes were incubated at 37 °C for 1 h with a horseradish-labeled secondary antibody (Beyotime Biotechnology, Shanghai, China), i.e., goat anti-rabbit IgG (A0208, 1:1000), donkey anti-goat IgG (number A0181, 1:1000) and goat anti-mouse IgG (A0216, 1:1000). Protein was detected using chemiluminescence, and images were captured using a Tanon-5200 system (Shanghai Tianneng Technology, Shanghai, China). Densitometry was performed.

2.8. Enzyme-linked immunosorbent assay (ELISA)

TNF-α and IL-6 concentrations in the hippocampus were detected using ELISA kits (catalog nos. xy-E11944 and xy-E11733, respectively; Shanghai Xinyu Biotech; Shanghai, China).

2.9. Detection of cleaved caspase-3 and assay of caspase-3 activity

The CA1 region of the hippocampus was excised (1.5 × 1.5 × 0.3 cm) and dehydrated through step-wise soaking in different concentrations of alcohol (50%, 70%, 85%, 95% and anhydrous ethanol). Tissues were embedded in paraffin and cut into ~5-μm thick sections. After deparaffinization and rehydration, 0.01 M sodium citrate buffer was added, and sections were placed in a microwave oven for 15 min for antigen retrieval. Sections were incubated with 3% H_2O_2 at room temperature for 1 h to eliminate endogenous peroxidase activity. After rinsing with PBS, the sections were incubated with normal rabbit serum (BIOSS, USA) at room temperature for 1 h. Sections were probed at room temperature for 1 h each with rabbit anti-cleaved caspase-3 antibody (1:50, ab13847, Abcam) followed by horseradish-labeled goat anti-rabbit IgG (1:1000, ab6721, Abcam). Sections were stained with DAB solution and counterstained with hematoxylin. Finally, the sections were put into xylene twice for 3 min each, sealed with a neutral runner, and placed into an oven at 65 °C for 15 min. Images were acquired by a camera on a Nikon microscope (Eclipse Ni), and the positively stained area was calculated.

Caspase-3 activity in the hippocampus was measured using a caspase-3 colorimetric assay kit (Jiangsu KeyGen Biotechnology, Nanjing, Jiangsu, China) per the manufacturer's instructions. Colorimetric detection was performed at 405 nm.

2.10. Statistical analysis

Data are presented as the mean ± standard deviation. Differences in neurological function scores were assessed using the Kruskal-Wallis H test. Differences in other variables were assessed using one-way analysis of variance followed by the Tukey test for multiple comparisons. Statistical significance was defined as a 2-sided $P < 0.05$.

3. Results

3.1. EA pretreatment at the GV20 acupoint improved neurological function following CA/CPR

We performed EA pretreatment on mice three days prior to CA/CPR induction to determine whether stimulation at the GV20 acupoint can protect against brain injury (Fig. 1A). Five neurological functions were assessed (Fig. 1B). CA/CPR significantly decreased the neurological function score compared to sham-operated animals. The neurological function deficit was significantly attenuated by EA pretreatment at the GV20 acupoint but not by pretreating at a nonacupoint.

EA pretreatment at the GV20 acupoint reduced cell apoptosis following CA/CPR.

To determine whether EA pretreatment could affect neuronal apoptosis following CA/CPR, the expression of cleaved caspase-3 was detected using immunohistochemistry, and caspase-3 activity was measured using a colorimetric assay (Fig. 2). The CA/CPR group showed the highest levels of cleaved caspase-3 expression and activity in hippocampal tissue. Pretreatment with EA led to significantly lower expression levels and activity compared with the CA/CPR group without pretreatment or the CA/CPR group pretreated at a non-acupoint.

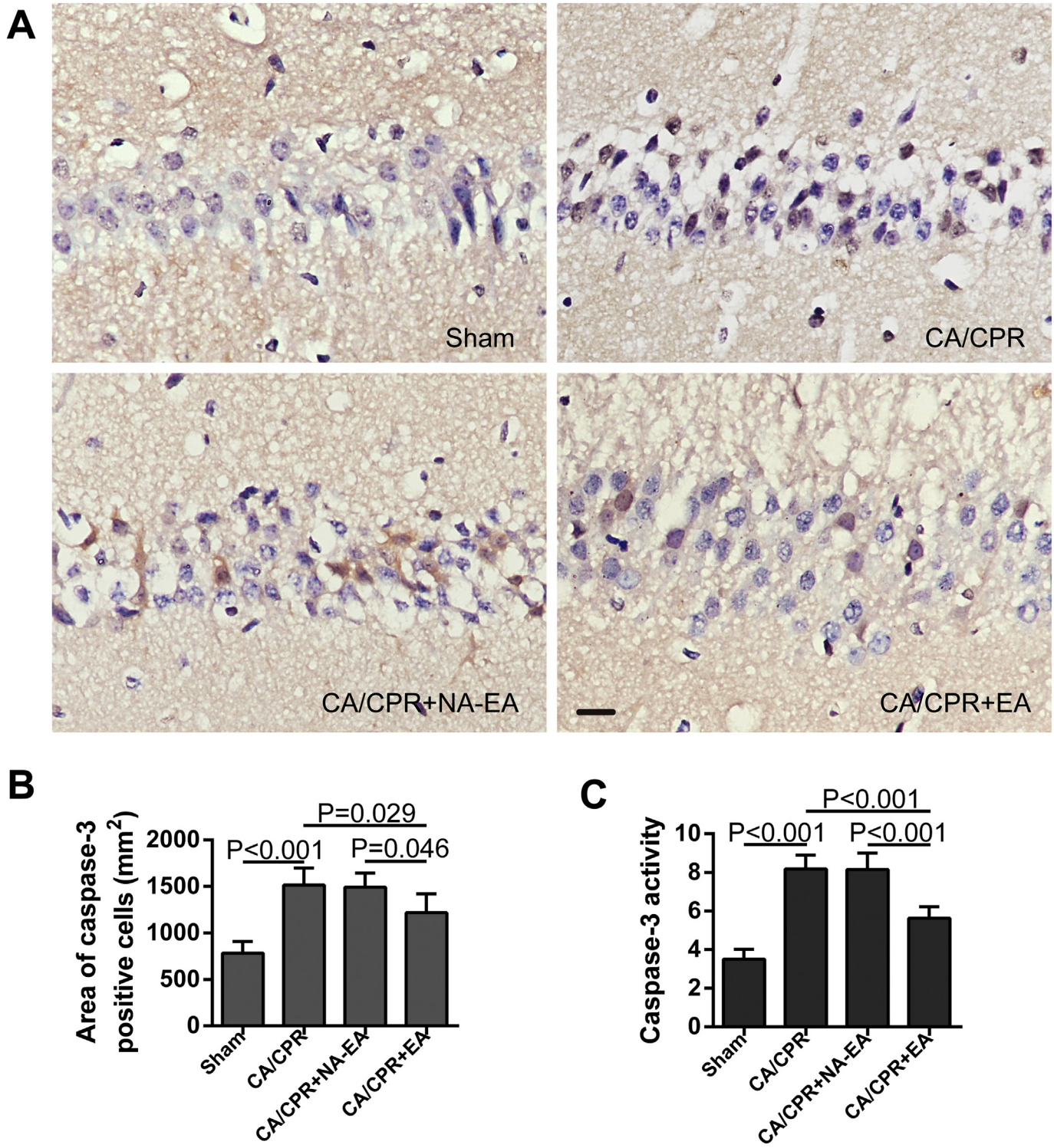

Fig. 2. EA pretreatment at the GV20 acupoint limits neuronal apoptosis and caspase-3 activity after CA/CPR (A) Representative images of cleaved caspase-3 immunohistochemistry in the hippocampus CA1 region from sham-operated or CA/CRP mice. Bar, 20 μm. (B) Quantification of positively stained caspase-3 cells in panel (A). (C) Quantification of caspase-3 activity in hippocampal tissue. Data are mean ± SD (n = 6 per group). Abbreviations: CA/CPR, cardiac arrest/cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at the GV20 acupoint; NA-EA, electroacupuncture pretreatment at a nonacupoint.

3.2. EA pretreatment at the GV20 acupoint alleviated oxidative stress damage and neuroinflammatory response following CA/CPR

Oxidative stress and an excessive inflammatory response are related to brain injury after cardiac arrest; therefore, we measured markers of both (Fig. 3). NOX2 and NOX4 mRNA and protein expression in the CA/CPR group was significantly higher than expression in the sham group. EA pretreatment at the GV20 acupoint markedly reduced NOX2 and NOX4 expression, while EA pretreatment at a nonacupoint did not. The CA/CPR group showed significantly higher hydrogen peroxide (H_2O_2) levels than the sham group, and this increase was eliminated by EA. Proinflammatory cytokines TNF-α and IL-6 were significantly lower in the CA/CPR group that received EA pretreatment at the GV20 acupoint compared with groups that received no EA or EA at a nonacupoint.

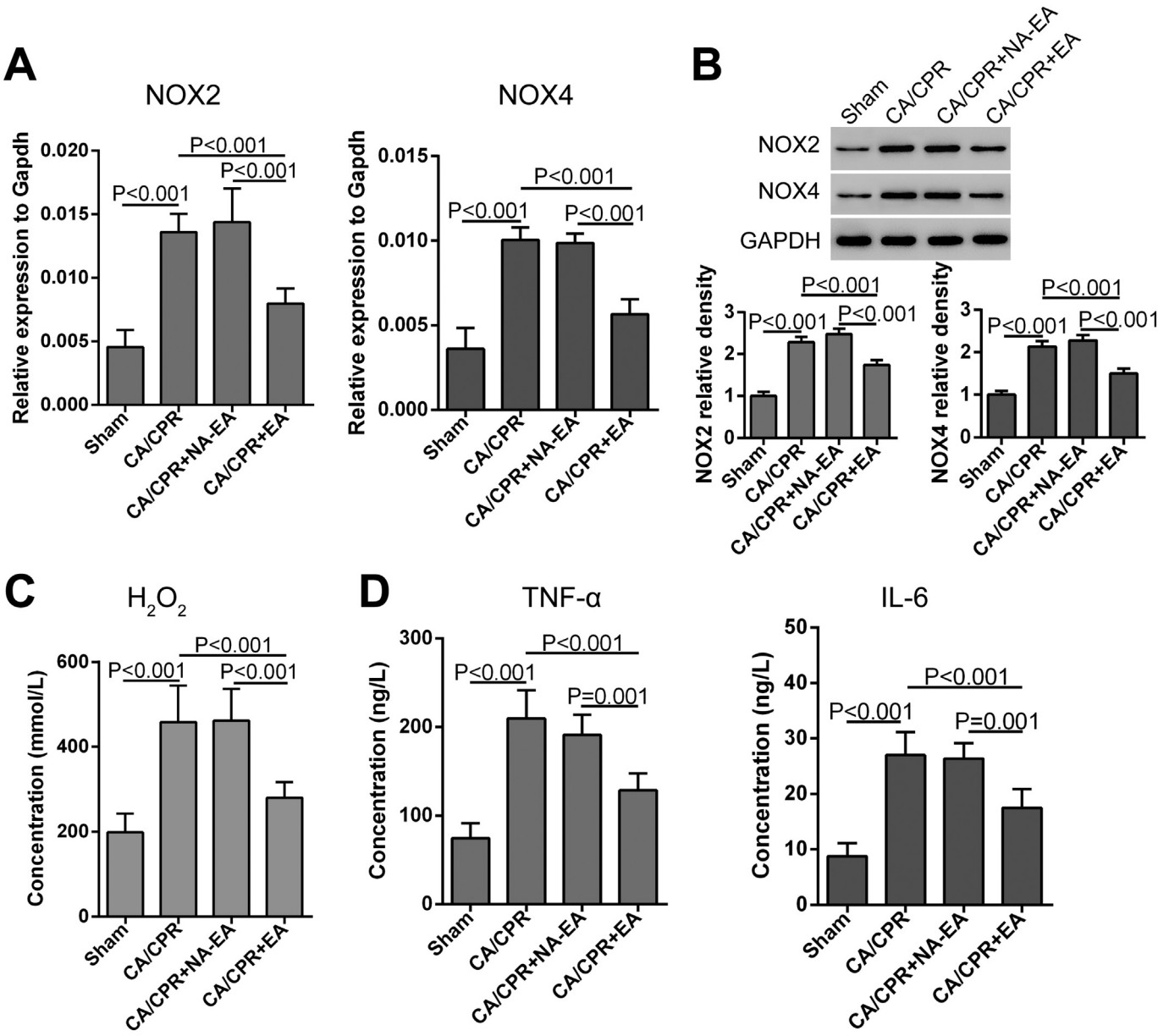

Fig. 3. EA pretreatment at the GV20 acupoint improves oxidative stress damage and excessive neuroinflammation following CA/CPR. NOX2 and NOX4 expression was measured in the hippocampus at the level of (A) mRNA and (B) protein. (C) H_2O_2 concentration in mouse serum. (D) TNF-α and IL-6 levels in the hippocampus as measured by enzyme-linked immunosorbent assay. Data are mean ± SD (n = 6 per group). Abbreviations: CA/CPR, cardiac arrest/cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at the GV20 acupoint; NA-EA, electroacupuncture pretreatment at a nonacupoint.

3.3. EA pretreatment activated AKT/eNOS signaling pathway after CA/CPR

NO and the AKT/eNOS signaling pathway exert neuroprotective effects in brain injury [15,20]. CA/CPR treatment significantly lowered NO production (Fig. 4A). EA pretreatment at the GV20 acupoint significantly increased NO production although not to the levels in the sham group.

To explore the possible molecular mechanisms of EA pretreatment, western blots were performed (Fig. 4B and C). The CA/CPR group showed lower levels of p-AKT, p-eNOS and total eNOS than the sham group. This effect was reversed in CA/CPR mice that received EA pretreatment at the GV20 acupoint. Total AKT levels did not vary significantly among the animal groups.

3.4. EA pretreatment at the GV20 acupoint acted via AKT/eNOS to protect against brain injury following CA/CPR

To determine whether EA pretreatment at the GV20 acupoint acts via the AKT/eNOS pathway to protect against brain injury, animals were given an AKT inhibitor (LY294002) or eNOS inhibitor (L-NIO) 30 min before EA treatment (Fig. 5A). LY294002 and L-NIO effectively blocked the AKT/eNOS signaling pathway (Fig. 5B and C). The inhibitors reverse the beneficial effects of EA pretreatment on neurological function following CA/CPR and also interfered with NO production (Fig. 5D and E).

Both inhibitors also reversed the EA-induced reduction in NOX2, NOX4, hydrogen peroxide (H_2O_2), TNF-α and IL-6 levels in CA/CPR mice (Fig. 6).

The inhibitors increased the levels of neural apoptosis after EA pretreatment to levels similar to those in the absence of EA (Fig. 7).

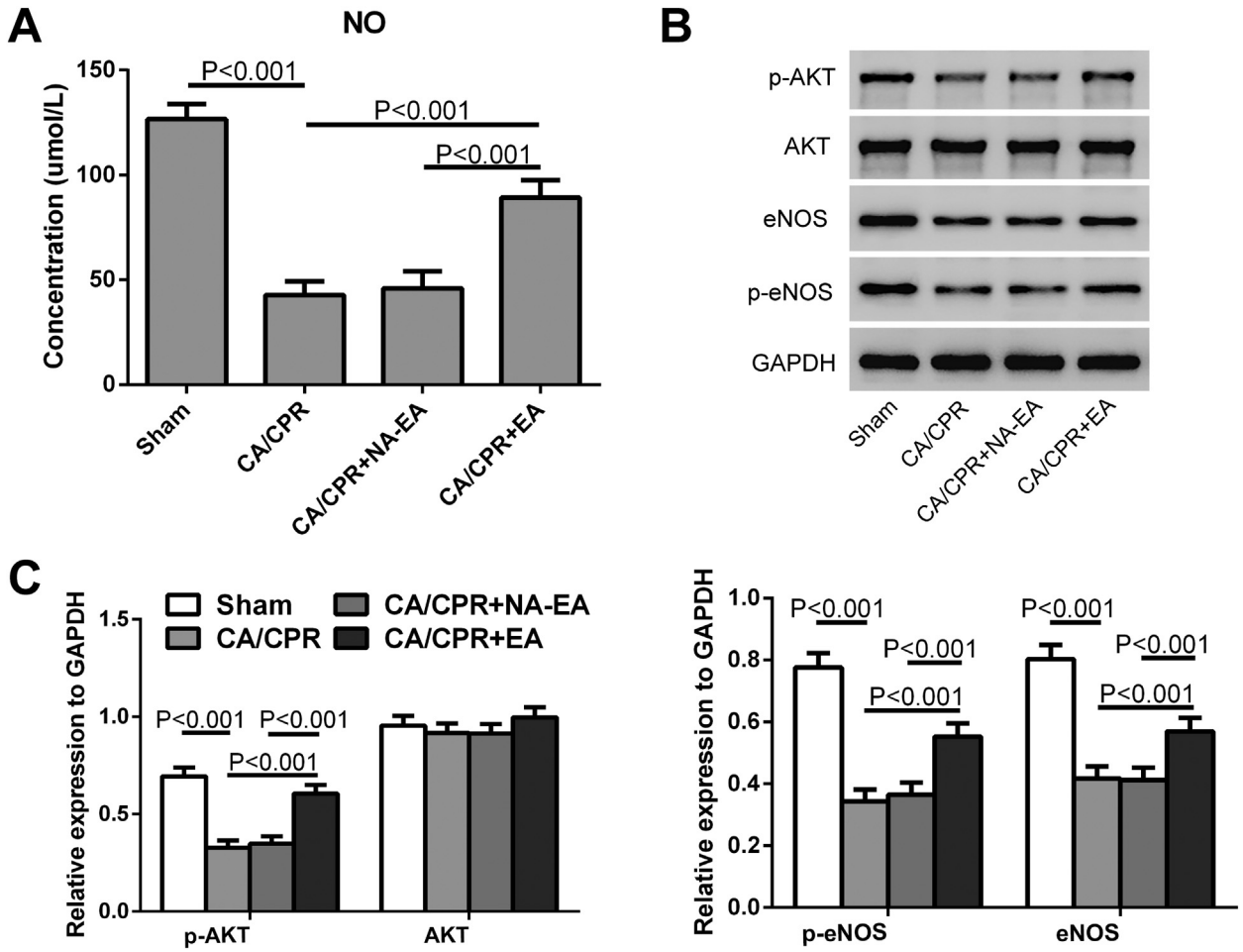

Fig. 4. EA pretreatment at the GV20 acupoint induces NO production by activating the AKT/eNOS signaling pathway. Mice were subjected to CA/CPR with or without EA pretreatment at the GV20 acupoint. Sham-operated animals served as controls. (A) NO concentration in mouse serum. (B) Western blot against AKT, p-AKT, eNOS and p-eNOS in all treatment groups. (C) Quantitative analysis of the western blots in panel (B). Data are mean ± SD (n = 6 per group). Abbreviations: CA/CPR, cardiac arrest/cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at the GV20 acupoint; NA-EA, electroacupuncture pretreatment at a nonacupoint; NO, nitric oxide.

Interestingly, LY294002 or L-NIO did not significantly alter the brain injury caused by CA/CPR itself.

4. Discussion

In this study, we found that EA pretreatment at the GV20 acupoint protected mice from brain injury after CA/CPR by reducing factors of oxidative stress and neuroinflammation and inhibiting neuronal apoptosis. The AKT/eNOS signaling pathway mediated this neuroprotection.

A growing amount of evidence confirms EA as an effective treatment for a wide variety of pain and diseases, such as neuro- and mental health-related diseases, gastrointestinal disorders, drug addiction, stress, and urinary incontinence [21–24]. For example, EA pretreatment protects against ischemia and reperfusion injury, promotes neurological recovery, and regulates brain activity through release of neuropeptides [25]. Several organizations worldwide have endorsed the efficacy and safety of EA [26–28]. These considerations led us to further examine the potential neuroprotective effects of EA pretreatment on brain injury in our CA/CPR model.

We established a modified mouse model of CA/CPR using chest compressions, supplemented oxygen and administration of epinephrine to establish a model that closely mimics the human version of CPR after cardiac arrest. We confirmed that our model induced brain injury based on depressed neurological function and increased oxidative damage, neuroinflammation, and neuronal apoptosis. These results are consistent with many previous studies [29–33], suggesting the relevance of our CA/CPR model.

When we pretreated our CA/CPR-treated animals with EA at the GV20 acupoint, we found increased neurological scores and decreased neuronal apoptosis, thus verifying the neuroprotective role of EA.

Oxidative stress, inflammation and apoptosis are associated with brain injury [34–36]. In our present study, EA pretreatment significantly attenuated these symptoms following CA/CPR. EA pretreatment also increased NO production, which modulates oxidative stress, improves mitochondrial function, limits neurological impairment, and improves survival rates after resuscitation following cardiac arrest [13,15,16]. Therefore, EA pretreatment might stimulate neuroprotective effects against injuries induced by CA/CPR.

Activation of AKT/eNOS signaling can protect against myocardial and cerebral ischemic injury [20,37,38]. The same signaling pathway may be involved in CA/CPR-induced injury since an AKT inhibitor and an eNOS inhibitor completely eliminated the protective effects of EA pretreatment. Western blots showed that EA pretreatment increased expression of p-AKT and p-eNOS in the CA/CPR model. Taken together, these results indicate that EA pretreatment acts via AKT/eNOS signaling to provide neuroprotection.

Our data are of high clinical relevance as most CA/CPR incidents occur during the perioperative period and most surgeries are scheduled. In addition EA has few side effects, is easy to perform, and is well received by most patients. Based on our data, we believe EA could

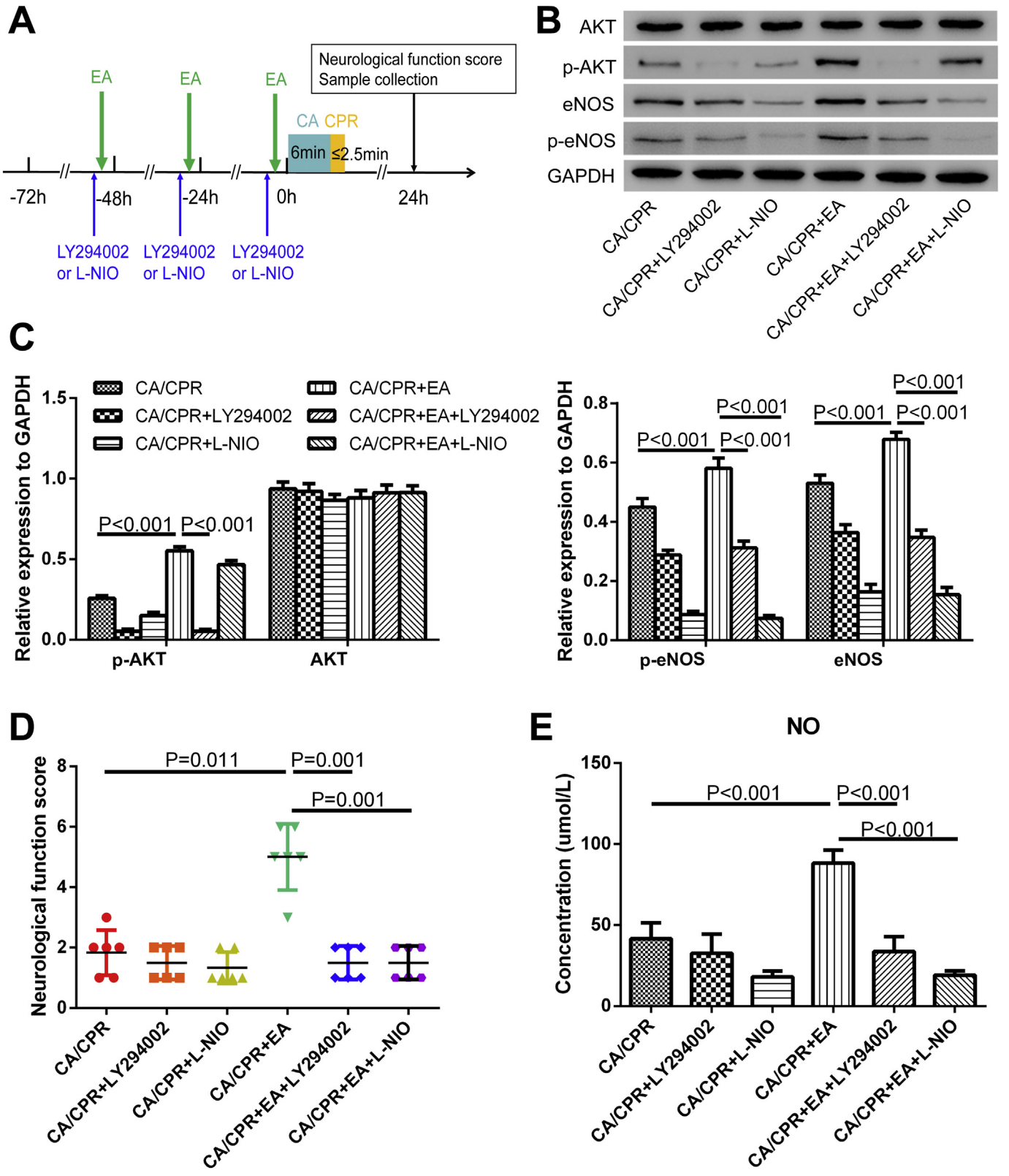

Fig. 5. Inhibition of the AKT/eNOS signaling pathway diminishes EA-induced neuroprotection. Mice were subjected to CA/CPR with or without EA pretreatment at the GV20 acupoint and with or without the AKT inhibitor LY294002 or the eNOS inhibitor L-NIO. (A) Schematic of experimental timeline. (B) Western blot expression of AKT, p-AKT, eNOS and p-eNOS from hippocampal tissue. (C) Quantitative analysis of western blots in panel (B). (D) Mean neurological function score of the mice 24 h after the procedure. (E) NO concentration in mouse serum. Data are mean ± SD (n = 6 per group). Abbreviations: CA, cardiac arrest; CPR, cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at the GV20 acupoint; NA-EA, electroacupuncture pretreatment at a nonacupoint; NO, nitric oxide.

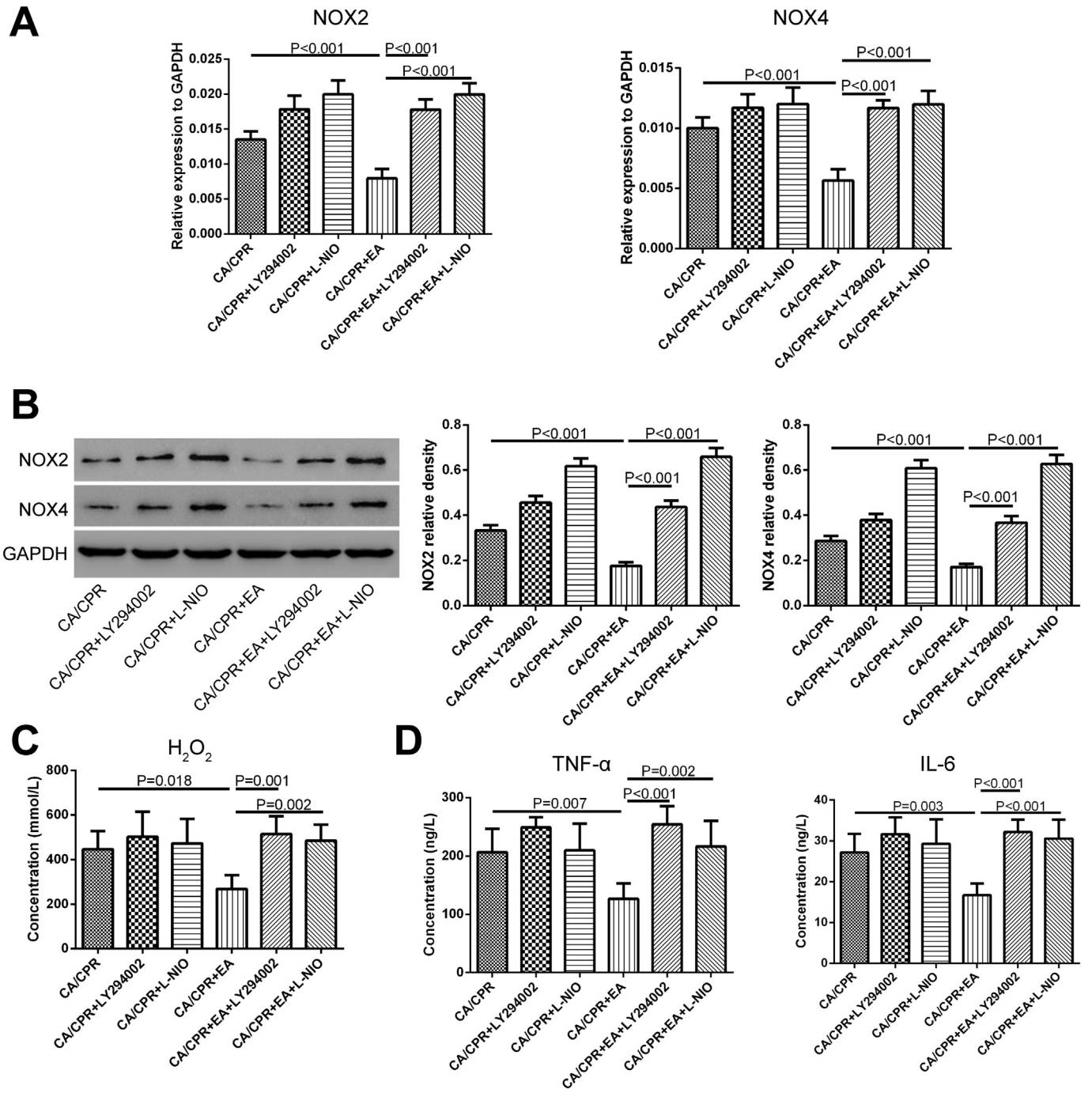

Fig. 6. Inhibition of the AKT/eNOS signaling pathway attenuates the beneficial effects of EA pretreatment on oxidative stress damage and the excessive neuroinflammatory response following CA/CPR. Mice were subjected to CA/CPR with or without EA pretreatment at the GV20 acupoint and with or without AKT inhibitor LY294002 or eNOS inhibitor L-NIO. NOX2 and NOX4 expression was measured in the hippocampus at the level of (A) mRNA and (B) protein. (C) H_2O_2 concentration in mouse serum. (D) TNF-α and IL-6 levels in the hippocampus. Data are mean ± SD (n = 6 per group). Abbreviations: CA/CPR, cardiac arrest/cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at the GV20 acupoint; NA-EA, electroacupuncture pretreatment at a nonacupoint.

represent a highly beneficial pretreatment for patients who undergo surgery, especially critical ill patients, by protecting against brain injury caused by perioperative cardiac arrest.

5. Conclusion

In conclusion, the present study provides evidence that EA pretreatment can effectively attenuate brain injury after CA/CPR through the AKT/eNOS signaling pathway. Our findings make it possible that acupuncture can be used as a pretreatment to alleviate brain damage caused by cardiac arrest during surgery. These data justify further investigations in animals and ultimately in humans.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lfs.2019.116821.

Author contributions

Yue Yong, Jun Guo, DongYu Zheng, Yonghua Li, Wei Chen, Jian Wang, Jian Wang, and Wenting Chen conducted the experiments. Ke Wang provided expertise and feedback. Yongqiang Wang and Yue Yong

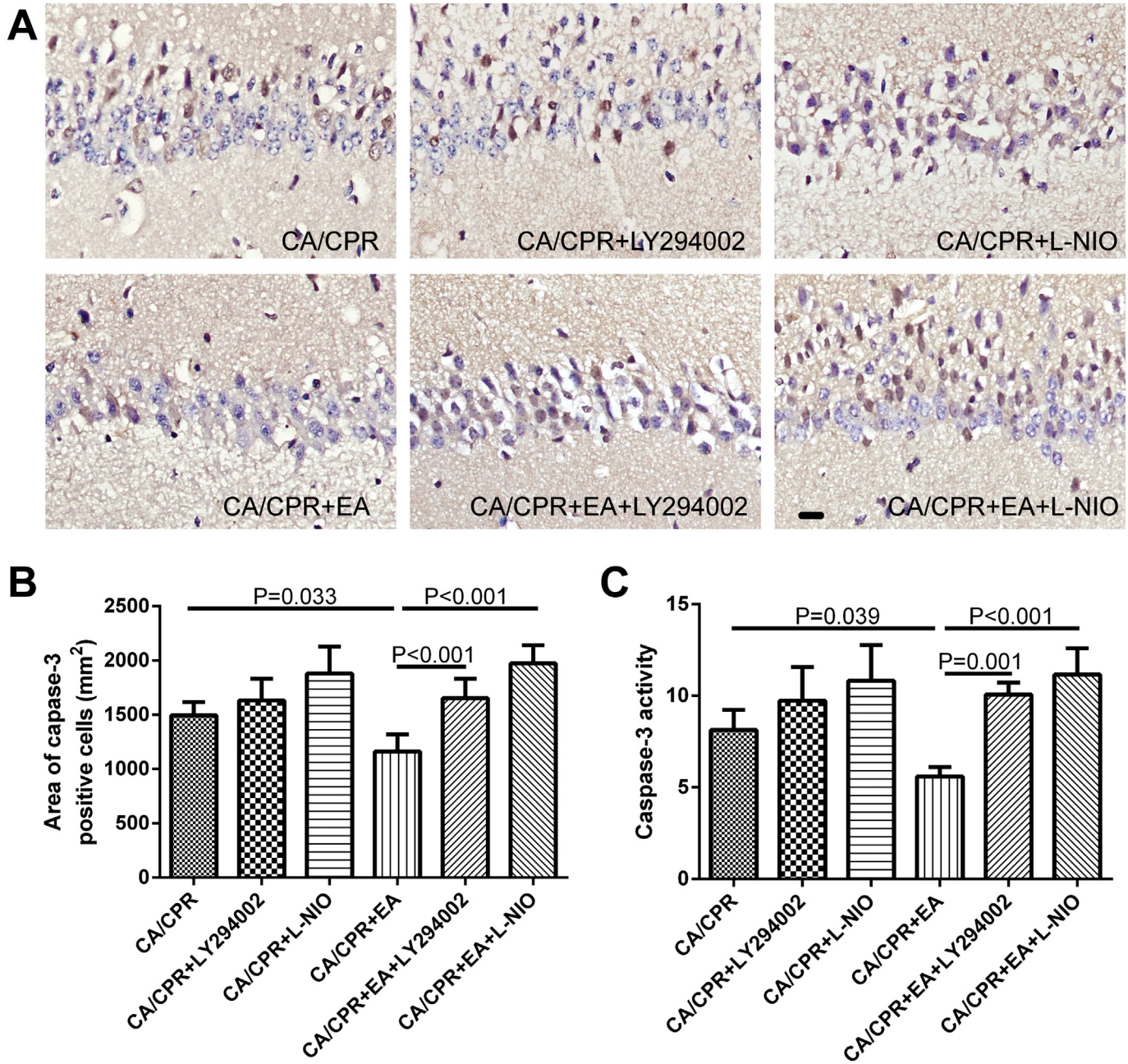

Fig. 7. Inhibition of the AKT/eNOS signaling pathway eliminates the beneficial effects of EA pretreatment on neuronal apoptosis following CA/CPR. Mice were subjected to CA/CPR with or without EA pretreatment at the GV20 acupoint and with or without AKT inhibitor LY294002 or eNOS inhibitor L-NIO. (A) Representative images of cleaved caspase-3 immunohistochemistry staining in the hippocampus CA1 region. Bar, 20 μm. (B) Quantification of positively stained caspase-3 cells from panel (A). (C) Caspase-3 activity in hippocampal tissue. Data are mean ± SD (n = 6 per group). Abbreviations: CA/CPR, cardiac arrest/cardiopulmonary resuscitation; EA, electroacupuncture pretreatment at the GV20 acupoint; NA-EA, electroacupuncture pretreatment at a nonacupoint.

wrote the manuscript, conceived the experiments and secured funding.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

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