Electroacupuncture Improves Survival in Rats with Lethal Endotoxemia *via* the Autonomic Nervous System

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ABSTRACT

Background: Recent advances have indicated a complex interplay between the autonomic nervous system and the innate immune system. Targeting neural networks for the treatment of sepsis is being developed as a therapeutic strategy. Because electroacupuncture at select acupoints can modulate activities of the autonomic nervous system, we tested the hypothesis that electroacupuncture at specific acupoints could modulate systemic inflammatory responses and improve survival *via* its impact on the autonomic nervous system in a rat model of sepsis.

Methods: Sprague-Dawley male rats received electroacupuncture for 45 min before and at 1, 2, or 4 h after a lethal dose of intraperitoneal lipopolysaccharide injection (6 mg/kg). Outcomes included survival and systemic cytokine responses. Also, the possible roles of neural circuitry, including the hypothalamic-pituitary-adrenal axis and the autonomic nervous system, were evaluated.

Results: Electroacupuncture pretreatment at the Hegu acupoints significantly attenuate systemic inflammatory responses and improve survival rate from 20% to 80% in rats with lethal endotoxemia. Such a site-specific effect requires the activation of muscarinic receptors in the central nervous system, but not increasing central sympathetic tone. In the periphery synergistic, rather than independent, action of the sympathetic and parasympathetic systems is also necessary.

Conclusions: Electroacupuncture pretreatment has a dramatic survival-enhancing effect in rats with lethal endotoxemia, which involves the activation of efferent neural circuits of the autonomic nervous system (*e.g.*, cholinergic antiinflammatory pathway). This approach could be developed as a prophylactic treatment for sepsis or perioperative conditions related to excessive inflammation.

What We Already Know about This Topic

- There are complex interactions between the autonomic nervous system and the innate immune system

What This Article Tells Us That Is New

- Rats pretreated with electroacupuncture at a specific acupoint that affects the efferent neural circuits of the autonomic nervous system attenuated their systemic inflammatory responses and improved their survival from lethal endotoxin administration

DESPITE more than 20 yr of extensive research and development, the incidence of sepsis and the number of sepsis-related deaths are rising. Depending upon the standards of medical care, mortality of sepsis could vary between 30% and 70%.[1,2] Sepsis is a heterogeneous, dynamic syndrome and involves a complex interplay of different biologic systems, most notably the immune system, the coagulation system, and the autonomic nervous system (ANS).[3] Recent advances in the field of neuroimmunology have shown that the ANS is one of the key pathways in the neuroimmune modulating network, and the balance between the two branches of the ANS (*e.g.*, sympathetic and parasympathetic) is important in directing the inflammatory response toward either pro- or antiinflammatory outcomes.[4] Consequently,

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methods that target the brain-to-immune neural circuits to control excessive immune responses while maintaining optimal host defense, such as transcutaneous vagus nerve stimulation and pharmacologic inhibition of sympathetic nervous system, are being developed for sepsis.[5,6]

Acupuncture is a 5,000-plus-year-old practice that is still widely used in many countries today.[7] Accumulating evidence has demonstrated that acupuncture at select acupoints can modulate activities of the ANS. For example, electroacupuncture at Neiguan (Pc-6; located in the groove caudal to the flexor carpi radialis and cranial to the superficial digital flexor muscles) significantly increases vagal activity, as measured by spectral analysis of heart rate variability.[8,9] In contrast, electroacupuncture at Hegu (Li-4; located at the junction of the first and second metacarpal bones) increases sympathetic tone, as evidenced by elevation of blood pressure and increased renal and adrenal nerve activities.[10,11] Based on these data, we tested the hypothesis that electroacupuncture at specific acupoints could inhibit systemic inflammatory responses and improve survival *via* its impact on the ANS in a rat model of sepsis (systemic administration of endotoxin).

Materials and Methods

Animals and Reagents

All studies were conducted in accordance with institutional animal care guidelines and approved by the Animal Care Committee of Shanghai Jiao Tong University (Shanghai, China). Adult male Sprague-Dawley rats (200–300g), provided by Sino-British SIPPR/BK Lab Animal Ltd. Co. (Shanghai, China), were housed at 22°C on a 12-h light/dark cycle.

Lipopolysaccharide (*Escherichia coli* 0111:B4), propranolol, phentolamine, clonidine, and mecamylamine were obtained from Sigma (St. Louis, MO). Atropine methyl nitrate was obtained from International Laboratory USA (San Bruno, CA). All test reagents were dissolved in physiologic saline.

Electroacupuncture Technique

The acupuncture points used in this study were Hegu (Li-4), located at the junction of the first and the second metacarpal bones, and Neiguan (Pc-6), located in the groove caudal to the flexor carpi radialis and cranial to the superficial digital flexor muscles. A set of nonacupoints located on the ulna side of the metacarpus served as controls. Stainless needles were inserted bilaterally to a depth of 5 mm and then held in place by plastic adhesive tape. Stimulation (current of 4 mA, alternating dense-and-disperse mode, 2 Hz [0.6-ms pulse width] *vs.* 100 Hz stimulation [0.2-ms pulse width], each lasting for 3 s) was delivered using an electrical stimulation device (HANS LH-202, Huawei Co. Beijing, China) for 45 min.

Chemical Sympathectomy

Rats received a subcutaneous injection of 100 mg/kg guanethidine sulfate (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan), dissolved in 0.9% NaCl, pH adjusted to 7.4, or vehicle of same volume for 2 consecutive days.[12]

Vagotomy

Under anesthesia and sterile condition, the right cervical vagus nerve of rats was exposed, ligated with a 4-0 silk suture, and transected. In sham-operated animals, the cervical vagus nerve was visualized, but was neither isolated from the surrounding tissues nor transected. Rats were allowed to recover for 4 days before the lipopolysaccharide challenge.

Splenectomy

Under anesthesia and sterile condition, the spleen of rats was identified through a midline laparotomy incision and removed using routine surgical technique. Sham animals received laparotomy without splenectomy. Nine days were allowed for recovery.

Experiment 1: Effect of Electroacupuncture on Survival

Under sodium pentobarbital anesthesia (50 mg/kg, intraperitoneal injection), rats received electroacupuncture 45 min before and at 1, 2, or 4 h after intraperitoneal lipopolysaccharide injection (6 mg/kg) at the following site: Hegu, Neiguan, or nonacupoints. A group of rats that received pentobarbital anesthesia and lipopolysaccharide, but not electroacupuncture, was included as a blank control. The survival rate was observed within the following 7 days. Each group included 20 rats. This experiment demonstrated superior protective efficacy of electroacupuncture at Hegu, relative to Neiguan and nonacupoints, but only before lipopolysaccharide challenge (fig. 1). Thus, electroacupuncture pretreatment at Hegu acupoints was chosen for subsequent experiments.

Experiment 2: Effect of Electroacupuncture on the Systemic Inflammatory Response

Rats received electroacupuncture at Hegu or electrostimulation at nonaccupoints before lipopolysaccharide challenge. A

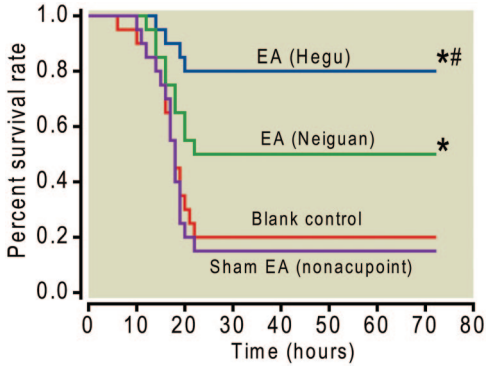

Fig. 1. Electroacupuncture pretreatment improves survival in lethal endotoxemia. SD rats received electroacupuncture for 45 min at Hegu (Li-4), Neiguan (Pc-6), or nonacupoints (sham) before a lethal dose of lipopolysaccharide (6 mg/kg, intraperitoneal injection). N = 20; * $P < 0.001$ *versus* blank control or electroacupuncture at nonacupoints; # $P < 0.05$ *versus* electroacupuncture at Neiguan. EA = electroacupuncture.

group of rats receiving lipopolysaccharide and anesthesia was included as additional controls. Serum pro-inflammatory cytokines (tumor necrosis factor (TNF) -α, interleukin (IL)-6, and IL-1β) and a prototype antiinflammatory cytokine (IL-10) were measured at 2, 4, and 6 h after lipopolysaccharide injection with ELISA (R&D Systems; Minneapolis, MN). At each time point, three groups as mentioned above (n = 6 or 10 per group) were included for obtaining blood samples.

Experiment 3: The Role of Hypothalamic-Pituitary-Adrenal Axis

The hypothalamic-pituitary-adrenal axis is one of the major gateways through which the central nervous system modulates the immune function, and provides an important physiologic feedback loop of inflammation through the antiinflammatory effects of corticosteroids.[4] To examine whether activation of hypothalamic-pituitary-adrenal axis is involved in the protective effect of electroacupuncture, serum corticosteroid was measured immediately after electroacupuncture pretreatment or electrical-stimulation at nonacupoints and at 2 h after lipopolysaccharide challenge. A group of rats receiving lipopolysaccharide and anesthesia and a group of rats receiving only anesthesia were included as additional controls (n = 10 per group). Corticosterone levels were determined using an immunoassay kit (R&D Systems).

Experiment 4: The Role of Sympathetic Nervous System

In this series of experiments, rats were treated with the following agents at 10 min before electroacupuncture at Hegu: centrally acting α_2-agonist clonidine (20 μg/kg, intraperitoneal injection), α-adrenergic antagonist phentolamine (1 mg/kg, intraperitoneal injection), β-adrenoceptor antagonist propranolol (5 mg/kg, intraperitoneal injection), or saline (n = 20 per group), and then subjected to lipopolysaccharide challenge. A separate group of rats received chemical sympathectomy before electroacupuncture and lipopolysaccharide challenge. Survival was the primary endpoint. In a separate experiment of the same design, serum TNF-α at 2 h after lipopolysaccharide was examined (n = 6 per group).

Experiment 5: The Role of Parasympathetic Nervous System

In this series of experiments, rats were treated with the following agents before electroacupuncture at Hegu: muscarinic receptor antagonist atropine methyl nitrate (5 μg/kg in 5 μl; intracerebroventricular injection under anesthesia; coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to midline, and 4.0 mm below the skull surface); nicotinic receptor antagonist mecamylamine (1 mg/kg; intraperitoneal injection); atropine methyl nitrate (1 mg/kg; intraperitoneal injection); or saline (intracerebroventricular injection as a control for atropine methyl nitrate; intraperitoneal injection as a control for mecamylamine or atropine methyl nitrate; n = 20 per group), and then subjected to lipopolysaccharide challenge. Of note, mecamylamine, at the dose 1 mg/kg used in this study, predominantly antagonizes peripheral and nonspecific nicotinic receptors,[13] and atropine methyl nitrate is unable to penetrate the blood-brain barrier.[14] Separate groups of rats receiving vagotomy, splenectomy, or sham surgery before the electroacupuncture pretreatment and lipopolysaccharide challenge were also included in this experiments (n = 20 per group). Survival was the primary endpoint. In a separate experiment of the same design, serum TNF-α was examined at 2 h after lipopolysaccharide challenge (n = 6 per group).

Statistical Analysis

Data are expressed as mean ± SD. In Experiments 2 and 3, comparison of serum inflammatory cytokines and corticosterone levels in the different treatment groups and different time points was carried out by using two-way ANOVA with the Tukey test. In Experiments 4 and 5, the differences of serum TNF-α at 2 h after lipopolysaccharide exposure among different treatment groups were analyzed using one-way ANOVA followed by *post hoc* Bonferroni *c*orrection for multiple comparisons. For survival analysis, Kaplan–Meier analysis was used followed by a log-rank test. P value <0.05 was considered statistically significant (two-tailed). All statistical analyses were performed by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).

Results

Electroacupuncture Pretreatment at Hegu Improved Survival in Rats with Lethal Endotoxemia

Three out of 20 rats receiving electroacupuncture treatment at nonacupoints before lipopolysaccharide challenge survived the endotoxemia (fig. 1). Electroacupuncture at Hegu before lipopolysaccharide challenge conferred dramatic protection: 16 of 20 rats survived the endotoxemia ($P < 0.0001$ *vs.* 4/20 in the blank control). No further dropouts within an observation period of 7 days indicates that electroacupuncture pretreatment conferred a lasting protection and did not merely delay the onset of death. Less protective effects were observed in rats receiving electroacupuncture at Neiguan before lipopolysaccharide challenge (survival rate: 10/20; $P = 0.049$ *vs.* Hegu). When delivered after lipopolysaccharide challenge, electroacupuncture treatment did not affect the survival rate at either Hegu or Neiguan.

Electroacupuncture Pretreatment Attenuated the Systemic Inflammatory Response to Lipopolysaccharide

Figure 2A displays that serum TNF-α was approximately 900 pg/ml at 2 h after the lipopolysaccharide challenge, and decreased to a level less than 60 pg/ml at 4 and 6 h. Such a temporal profile is consistent with previous reports.[15] Electroacupuncture pretreatment at Hegu (but not electro-stimulation at nonaccupoints) significantly decreased serum

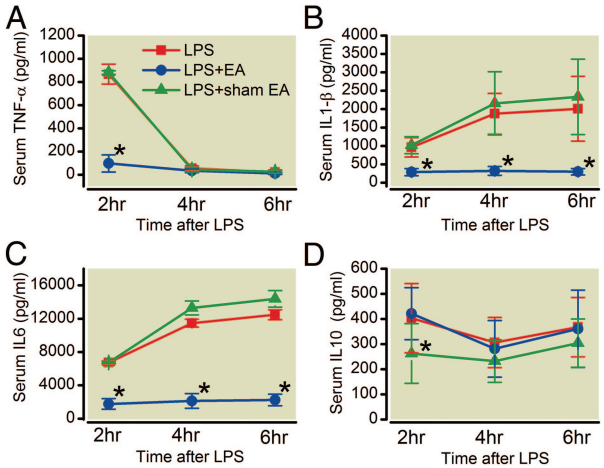

Fig. 2. Pretreatment of electroacupuncture at Hegu decreases the serum pro-inflammatory cytokines (*e.g.*, tumor necrosis factor-α, interleukin-6, interleukin-1β), but does not affect the antiinflammatory cytokine IL-10 in rats receiving lipopolysaccharide. After receiving electroacupuncture at Hegu or nonacupoints (sham), rats were injected with lipopolysaccharide (6 mg/kg, intraperitoneal injection); blood was collected 2, 4, and 6 h afterward. Serum tumor necrosis factor-α (*A*), interleukin-1β (*B*), interleukin-6 (*C*), and interleukin-10 (*D*) were measured using commercially available ELISA kits. Data are mean ± SD. For the time point of 2 h, n = 10 per group; for 4 and 6 h, n = 6 per group. * $P < 0.001$ *versus* LPS alone. EA = electroacupuncture; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-10 = interleukin-10; LPS = lipopolysaccharide; TNF-α = tumor necrosis factor-α.

TNF-α level at 2 h after lipopolysaccharide challenge ($P < 0.0001$). TNF-α level at 4 ($P = 0.23$) and 6 h was not affected ($P = 0.11$). Figure 2B displays that serum IL-1β was approximately 1,000 pg/ml at 2 h after the lipopolysaccharide challenge, and reached a plateau at 4 h after lipopolysaccharide challenge. Electroacupuncture pretreatment at Hegu significantly decreased IL-1β throughout the entire observation period ($P < 0.0001$). Figure 2C displays that serum IL-6 was approximately 6,500 pg/ml at 2 h after the lipopolysaccharide challenge, and reached a plateau at 4 h after lipopolysaccharide challenge. Electroacupuncture pretreatment at Hegu significantly decreased IL-6 throughout the entire observation period ($P < 0.0001$). Figure 2D displays that the prototype antiinflammatory cytokine IL-10 was not affected by electroacupuncture pretreatment ($P = 0.76$, 0.45, and 0.63, respectively).

The Protective Effect of Electroacupuncture Pretreatment Could Not Be Attributed to the Activation of Hypothalamic-Pituitary-Adrenal Axis

Serum corticosteroid in rats receiving lipopolysaccharide challenge was significantly decreased, rather than increased, by electroacupuncture pretreatment at Hegu as well as at nonacupoints ($P < 0.0001$ for both; fig. 3). Electroacupuncture at Hegu or nonacupoints alone also significantly decreased serum corticosteroid levels in rats not exposed to lipopolysaccharide ($P < 0.0001$ for both; fig. 3). Thus, the beneficial effect of electroacupuncture is not associated with increasing corticosteroid levels.

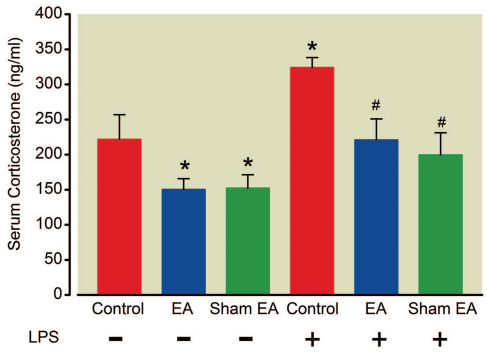

Fig. 3. Effects of electroacupuncture (EA) pretreatment *per se* or electroacupuncture pretreatment plus lipopolysaccharide (LPS) on serum corticosterone levels. Electroacupuncture pretreatment does not increase serum corticosteroid in rats exposed to LPS. In control rats not exposed to LPS, electroacupuncture at either Hegu or nonacupoints (sham) significantly decreases serum corticosterone levels. Data are mean ± SD, n = 10 per group. * $P < 0.05$ *versus* control rats not receiving LPS; # $P < 0.05$ *versus* LPS alone.

Peripheral Sympathetic Nervous System and Particularly β-adrenoceptors, but Not Increased Central Sympathetic Tone, Are Necessary for the Protective Action of Electroacupuncture

Consistent with a previous study of sympathectomy with 6-hydroxydopamine in an animal model of thermal injury with sepsis,[16] ablation of the sympathetic nervous system with guanethedine before lipopolysaccharide challenge significantly increased survival rate (10/20 *vs.* 3/20 in the control rats; $P = 0.025$) and attenuated serum TNF level ($P < 0.0001$). However, electroacupuncture pretreatment at the Hegu did not confer further protection in sympathectomized rats (survival rate: 8/20 *vs.* 10/20 in sympathectomized rats without electroacupuncture; $P = 0.66$; fig. 4A). Also, serum TNF level did not differ between sympathectomized rats with or without electroacupuncture pretreatment ($P = 0.49$; fig. 4B). Pretreatment with the β-antagonist propranolol (survival rate: 3/20; $P < 0.0001$; TNF-α: $P < 0.0001$), but not the α-antagonist phentolamine (survival rate: 16/20; $P = 0.67$; TNF-α: $P = 0.55$; figs. 4C and D) completely abolished the effects of electroacupuncture on survival and serum TNF. Somewhat surprisingly, pretreatment with clonidine, a centrally acting α_2-agonist that decreases central sympathetic tone,[17] did not block the effects of electroacupuncture (survival rate: 18/20; $P = 0.44$; TNF-α: $P = 0.68$; figs. 4E and F).

Central Muscarinic Receptor, Vagus Nerve, Peripheral Nicotinic Receptor, and Spleen Are Required for the Protective Action of Electroacupuncture

The vagus nerve has recently been identified as a major pathway through which immune function is regulated by the

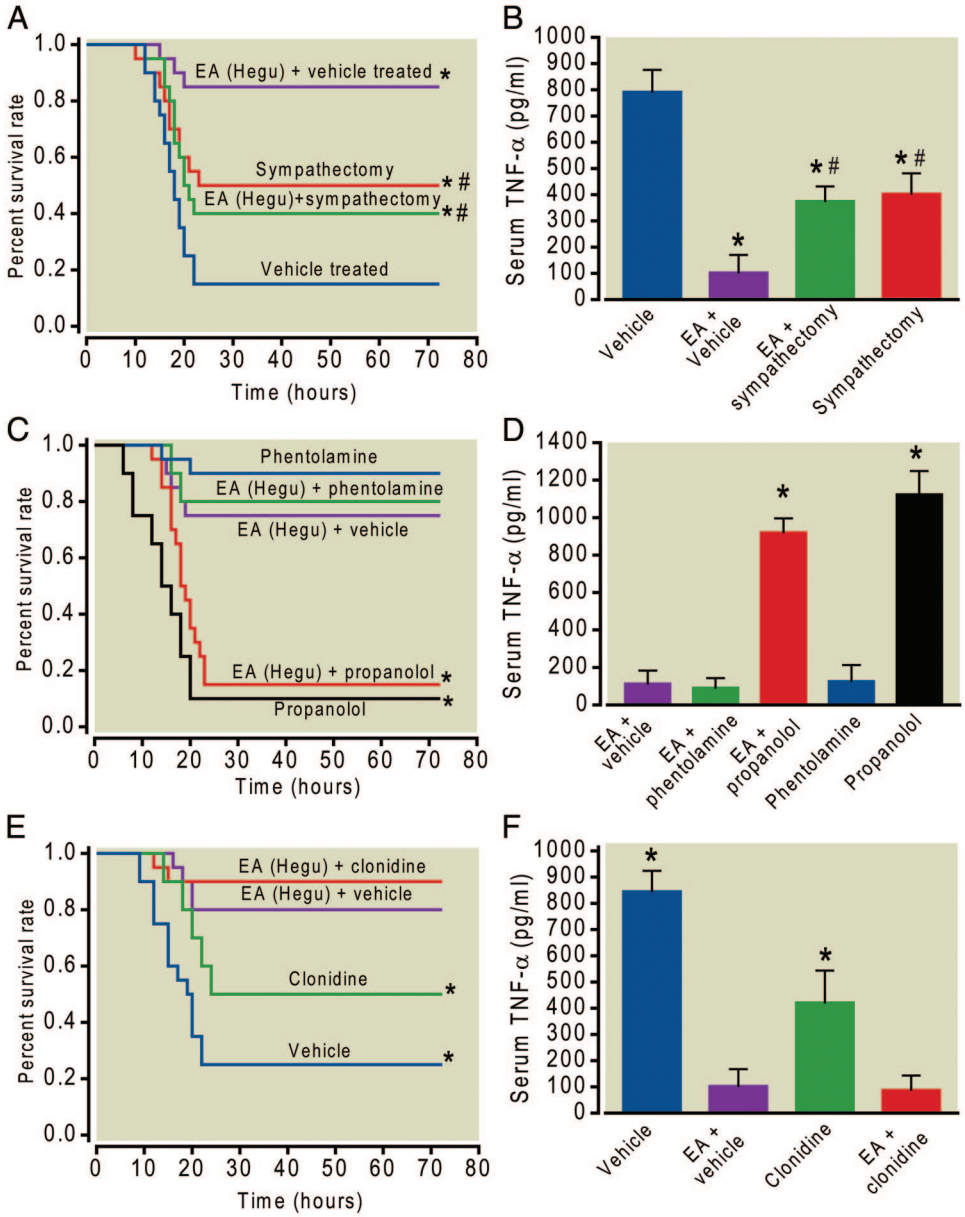

Fig. 4. Peripheral sympathectomy or β-adrenoceptor blockade, but not a central sympatholytic, abrogates the protective effect of electroacupuncture. Data are survival rate (n = 20 per group) and serum tumor necrosis factor-α level (n = 6 per group). (*A*, *B*) SD rats received guanethidine (100 mg/kg, subcutaneous injection, for 2 consecutive days) or vehicle. On the morning of the third day, rats were pretreated with electroacupuncture, and then received lipopolysaccharide (6 mg/kg, intraperitoneal injection). * $P < 0.01$ *versus* vehicle alone; # $P < 0.05$ *versus* electroacupuncture plus vehicle. (*C*, *D*) Propranolol (5 mg/kg, intraperitoneal injection), but not phentolamine (1 mg/kg, intraperitoneal injection), abolished the protective effect of electroacupuncture. * $P < 0.0001$ *versus* electroacupuncture plus vehicle control. (*E*, *F*) Intraperitoneal injection of clonidine, a centrally acting α_2-adrenoceptor agonist (20 μg/kg, dissolved in saline), does not affect the protective effect of electroacupuncture. * $P < 0.01$ *versus* electroacupuncture plus vehicle control. EA = electroacupuncture; TNF-α = tumor necrosis factor-α.

central nervous system, which is termed the "cholinergic antiinflammatory pathway."[18] Our results indicated that unilateral (right-sided) cervical vagotomy before lipopolysaccharide challenge significantly attenuated the protective effects of electroacupuncture pretreatment (survival rate: 7/20 *vs.* 16/20 in sham-operated rats; $P = 0.005$; TNF-α: $P < 0.0001$; figs. 5A and B). Systemic treatment with the nicotinic antagonist mecamylamine (survival rate: 4/20; $P < 0.001$; TNF-α: $P < 0.001$), but not the muscarinic antagonist atropine methyl nitrate (survival rate: 19/20; $P = 0.32$; TNF-α: $P = 0.65$; figs. 5C and D), completely blocked the protective effects of electroacupuncture. Atropine methyl nitrate delivered directly into the brain completely blocked the protective effects of electroacupuncture (survival rate of 6/20 *vs.* 15/20 in vehicle controls; $P = 0.007$; TNF-α: $P < 0.0001$; figs. 5E and F). These results are consistent with an

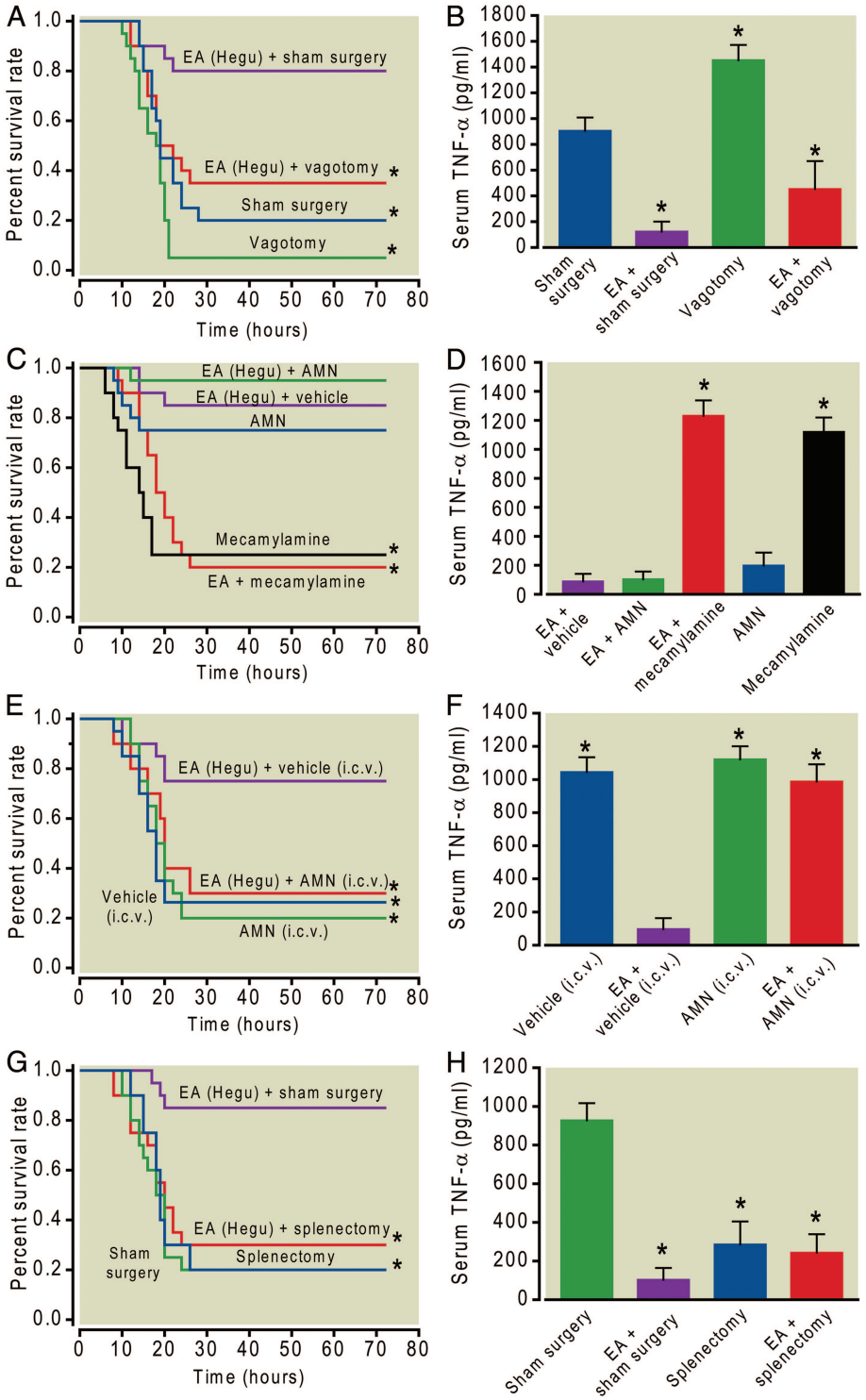

Fig. 5. Central muscarinic receptor, vagus nerve, peripheral nicotinic receptor, and spleen are required for the protective action of electroacupuncture at Hegu. Data are survival rate (n = 20 per group) and serum tumor necrosis factor-α level (n = 6 per group). (*A*, *B*) Cervical vagotomy *versus* sham surgery, at 4 days before lipopolysaccharide injection (6 mg/kg, intraperitoneal injection). * $P <$ 0.01 *versus* electroacupuncture plus sham surgery. (*C*, *D*,) Pretreatment with the nicotinic receptor antagonist mecamylamine (1 mg/kg, intraperitoneal injection) *versus* the muscarinic receptor antagonist atropine methyl nitrate (1 mg/kg, intraperitoneal injection). * $P <$ 0.001 *versus* electroacupuncture plus vehicle control. (*E*, *F*) Atropine methyl nitrate (5 μg/kg), delivered *via* the intracerebroventricular route at 15 min before electroacupuncture pretreatment. * $P <$ 0.001 *versus* electroacupuncture plus vehicle control. (*G*, *H*) Surgical ablation of the spleen * $P <$ 0.001 *versus* sham surgery. AMN = atropine methyl nitrate; EA = electroacupuncture; i.c.v. = intracerebroventricular; TNF-α = tumor necrosis factor-α level.

important role of the central muscarinic receptors in modulating peripheral cytokine production.[19] Recent work on the anatomical basis of the cholinergic antiinflammatory pathway indicates that the spleen is required for vagus control of inflammation, although the splenic nerve is classified as catecholaminergic.[20] In our study, although splenectomy *per se* significantly reduced serum TNF-α level compared with sham surgery group ($P < 0.0001$; fig. 5H), which is consistent with a previous study, [21] it did not improve the survival rate of rats challenged with a lethal dose of lipopolysaccharide ($P = 0.77$; fig. 5G). When electroacupuncture was applied to splenectomized animals, its survival-enhancing effect disappeared (survival rate of 6/20 *vs.* 17/20 in sham surgery controls; $P < 0.0001$; fig. 5G). Also, electroacupuncture failed to inhibit serum TNF-α level in splenectomized animals, unlike in intact animals ($P = 0.50$; fig. 5H).

Discussion

The current study for the first time demonstrated that electroacupuncture pretreatment at the Hegu acupoints can dramatically improve survival in rats with lethal endotoxemia. The production of proinflammatory cytokines (TNF-α, IL-6 and IL-1β) was also significantly attenuated, but antiinflammatory cytokine serum IL-10 was not affected.

Our study also demonstrated selectivity of the acupuncture: electroacupuncture at Hegu acupoints produced more powerful protection than electroacupuncture at Neiguan acupoints; electrostimulation at nonacupoints was not effective at all. To our knowledge, the site-specific effects of acupuncture, *i.e.*, "true" treatment acupoints with selective physiologic effects, have not been fully validated or accepted. A few influential clinical trials of migraine headache failed to show significant difference between acupuncture at select acupoints *versus* sham acupuncture.[22,23] In the current study, the use of anesthesia during electroacupuncture excluded some likely nonspecific physiologic effects caused by electrostimulation procedure, such as pain and immobilization stress, and thus provided more solid evidence for the existence of site-specific effects.

The timing of electroacupuncture treatment seems critical for the "rescue" effect in our study, because electroacupuncture must be delivered before lipopolysaccharide injection to produce a protective action. We speculate that the explicit requirement for pretreatment may be specific to the lipopolysaccharide model used in this study: immune pathology of endotoxemia models using bolus injection is characterized by a very rapid and overwhelming innate immune response that is very difficult to stop once it is in motion. Although bacterial infection models do not recapitulate many important features of human sepsis, they can provide important insights into mechanisms of the host response to pathogens.[24]

During the past decade, the immunomodulatory efficacy of acupuncture has been supported by increasing number of randomized controlled clinical trials for a number of immune- or inflammatory-related diseases, such as allergic asthma,[25] childhood persistent allergic rhinitis,[26] and rheumatoid arthritis.[27,28] Animal studies have also indicated that acupuncture pretreatment has protective effects against endotoxin-induced acute lung and kidney injuries.[29,30] However, little is known about its biologic basis.

In this study, we found that electroacupuncture at Hegu does not enhance the release of glucocorticoids or antiinflammatory cytokines (*e.g.*, IL-10), suggesting that humoral pathways are not responsible for the immunomodulatory efficacy of electroacupuncture. Instead, such an effect requires the participation of muscarinic receptors in the central nervous system, but not increasing central sympathetic tone. Synergistic, rather than independent, action of peripheral sympathetic and parasympathetic systems is also necessary. At the first glance, these results seem difficult to explain or discriminate the roles of sympathetic and parasympathetic components. However, newly identified cholinergic antiinflammatory pathway suggests that the long-standing attempt to separate the neural circuitry that controls immune responses into discrete sympathetic and parasympathetic components is imprecise. On the contrary, at least in the periphery, the involvements of sympathetic *versus* parasympathetic systems are not independent, either anatomically or functionally.[18] From a systematic perspective, our data strongly support this important conceptual advance. We now put forward the following framework mainly based on the cholinergic antiinflammatory pathway to explain our findings: electroacupuncture at Hegu activates a brain muscarinic receptor-mediated network possibly through somatoautonomic reflexes,[31,32] and subsequently increases vagus nerve activity. The vagus nerve terminates in synaptic-like structures around principal cells of the celiac-superior mesenteric plexus ganglia, a site where catecholaminergic splenic fibers originate. *Via* two serially connected neurons in these ganglia, vagus nerve modulates the activities of splenic nerve through nicotinic acetylcholine receptor.[33] Increased norepinephrine released by the splenic nerve then acts on β-adrenergic receptors expressed on B and T cells of the spleen to produce acetylcholine.[34,35] Enhanced acetylcholine levels in the spleen then activates nicotinic receptor expressed on macrophages to inhibit proinflammatory cytokine release.

In addition, since it has been suggested that lipopolysaccharide-induced hemodynamic instability contributes to mortality,[36] a paradoxical hypertensive response mediated by stimulation at Hegu acupoints might explain electroacupuncture's survival-enhancing effect. However, both previous studies[10,11] and our preliminary data indicated that the pressor response elicited by electroacupuncture at Hegu acupoints lasts for only 2 or 3 min after cessation of the stimulation. Also, if the protective effect is mediated by pressor effect, electroacupuncture delivered after lipopolysaccharide injection should have been more effective; however, it's

clearly not the case in this study. Thus, hemodynamic effect produced by electroacupuncture cannot account for its survival-enhancing effect.

In conclusion, electroacupuncture pretreatment at the Hegu acupoints inhibited systemic inflammatory responses and enhanced survival in rats with lethal endotoxemia. The underlying mechanism involves the activation of efferent neural circuits of the ANS, *e.g.*, the cholinergic antiinflammatory pathway. These findings encourage the development of electroacupuncture as a prophylactic treatment for sepsis or other perioperative conditions related to excessive inflammation.

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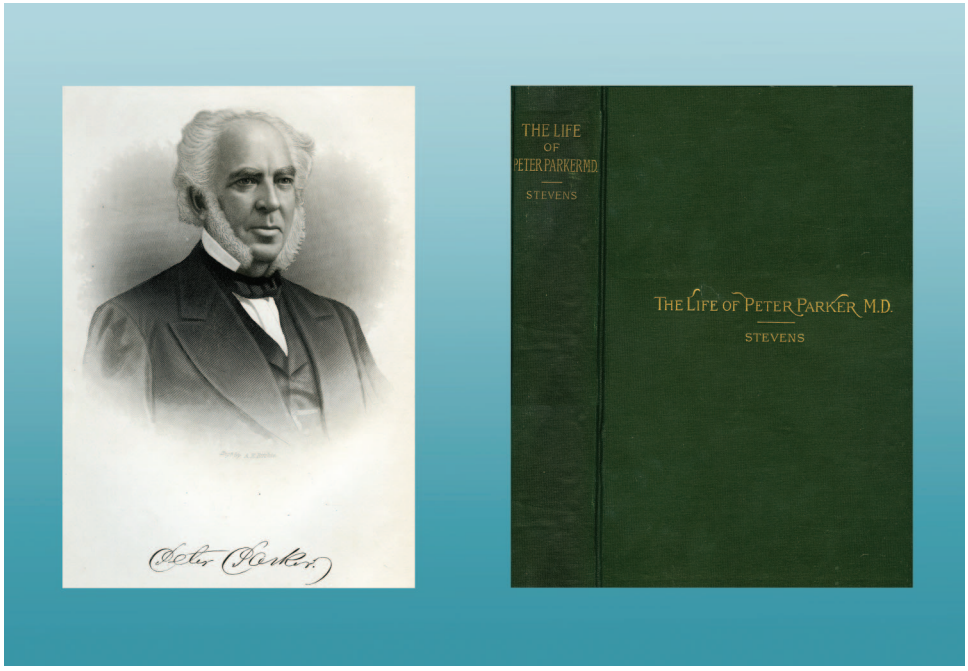
ANESTHESIOLOGY REFLECTIONS

The Ethereal Peter Parker, M.D., D.D.



"Peter Parker" is regarded as the alter ego of the fictional character Spiderman by many younger physicians; however, older professionals may recognize "Peter Parker" as the name of the Presbyterian medical missionary who introduced surgical anesthesia to China. Indeed, Rev. Dr. Parker (1814–1888) was etherizing Chinese patients for surgery as early as the summer of 1847 and was chloroforming others by the following year. In later years, Parker's likeness (*left*) was captured by engraver A. H. Ritchie. In 1896 a Yale professor named Rev. George B. Stevens, M.D., published (*right*) a 356-page biography, *The Life, Letters, and Journals of the Rev. and Hon. Peter Parker, M.D., Missionary, Physician, and Diplomatist: The Founder of Medical Missions and Founder of the Ophthalmic Hospital in Canton*. Biographer Stevens' reference to Parker as "diplomatist" reminds readers that the missionary also served on diplomatic missions as an interpreter on behalf of U.S. Presidents Tyler and then Pierce in negotiating America's first treaty with China. (Copyright © the American Society of Anesthesiologists, Inc. This image also appears in the Anesthesiology Reflections online collection available at www.anesthesiology.org.)

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