

Gastric electrical stimulation improves enteric neuronal survival

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Abstract. Research and clinical experience with vagotomy have confirmed that damage to the central nervous system severely affects physiological movement in the gastrointestinal system. The aim of this study was to investigate the effects of synchronized dual-pulse gastric electrical stimulation (SGES) on the apoptosis of enteric neurons and the possible pathways involved in these effects in vagotomized rats. For this purpose, Male Sprague-Dawley (SD) rats were randomized into a control group, an early subdiaphragmatic vagotomized group (ESDV group), an early subdiaphragmatic vagotomized group with short-term SGES (ESDV + SSGES group), a terminal subdiaphragmatic vagotomized group (TSDV group) and a terminal subdiaphragmatic vagotomized group with long-term SGES (TSDV + LSGES group). The expression levels of connexin 43 (Cx43), glial cell line-derived neurotrophic factor (GDNF), p-Akt, pan-Akt and PGP9.5 were assessed by RT-qPCR, western blot analysis and immunofluorescence staining. Apoptosis was determined by terminal-deoxynucleotidyl transferase-mediated nick-end labeling (TUNEL) assay. We found that Cx43 expression was decreased in the ESDV and TSDV groups, but was significantly upregulated in the SSGES and LSGES groups. In addition, the GDNF and PGP9.5 expression levels were significantly decreased in the ESDV group compared with the control and TSDV groups and were upregulated in both the SSGES and LSGES groups. The LSGES group exhibited a clear increase in p-Akt expression compared with the TSDV group. Fewer TUNEL-positive cells were observed in the SSGES and LSGES groups than in the ESDV and TSDV groups. More TUNEL-positive cells were found in the stomach of rats subjected to subdiaphragmatic vagotomy. On the whole, our data indicate that SGES improved enteric neuronal survival, possibly through GDNF and the phosphatidylinositol 3-kinase (PI3K)/Akt pathways.

Introduction

The central nervous system, through the sympathetic and parasympathetic pathways, regulates the function of the enteric nervous system (ENS); the latter mainly governs the signaling from the central nervous system to the ENS following gastrointestinal injury. The ENS can function independently of the central nervous system via the myenteric and submucosal plexuses. A recent demonstrated that atrial fibrillation ablation leads to the functional impairment of gastric emptying, which may be mediated by damage to the vagal nerve (1). Vagal nerve stimulation increases enteric glial activation, which protects the intestines from burn-induced injury (2). The stomach is dominated by the vagal nerve, and its sensory information is transmitted via the afferent vagal nerve to the nucleus tractus solitarius, which modulates sensory information from multiple locations throughout the central nervous system (3).

Glial cell line-derived neurotrophic factor (GDNF) is a growth factor that promotes neuronal survival and differentiation, and markedly enhances nerve regeneration following severe nerve damage (4). GDNF also plays a protective role in hepatic steatosis in mice by decreasing liver fat content (5). GDNF regulates the phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) and extracellular signal-regulated kinase 1/2 (ERK1/2/mitogen-activated protein kinase (MAPK) pathways. These signals modulate cellular survival via the PI3K/Akt pathway and neuronal differentiation via the ERK1/2 MAPK pathway. Evidence indicates that GDNF and the PI3K/Akt signaling pathway are involved in enteric neuropathy (6,7), and electroacupuncture at acupoint ST-36 leads to enteric neuronal regeneration through GDNF and the PI3K/Akt signaling pathway in the diabetic rat colon (8). Nevertheless, limited information is available on the release of GDNF and its downstream pathway in the stomachs of vagotomized rats.

In recent years, increasing attention has been paid to gastric electrical stimulation (GES), a promising alternative treatment for functional gastrointestinal disorders. GES, when applied with optimal parameters, has an analgesic effect on visceral pain through the opioid system and the inhibition of spinal afferent neuronal activity (9). In a previous study, GES using trains of short pulses was shown to attenuate apomorphine-induced emetic responses via the activation of the amygdala (10). In addition, GES has been shown to alleviate symptoms (such as nausea, loss of appetite and early satiety) in 75% of patients with refractory gastroparesis (11). Of note, GES, when applied

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with the appropriate parameters, can inhibit gastric motility in healthy dogs, representing a potential treatment for obesity (12).

However, the effect of synchronized dual-pulse GES (SGES) on the communication between enteric glial cells (EGCs) and enteric neurons and the possible involvement of GDNF with enteric neurons through the PI3K/Akt pathway in a vagotomized rat stomach are unknown. Thus, the aim of this study was to evaluate the effects of SGES on the apoptosis of enteric neurons, and to further investigate whether GDNF and the PI3K/Akt pathway are involved in the communication between EGCs and enteric neurons.

Materials and methods

Animals. Male Sprague-Dawley rats weighing 250–350 g were obtained from the Experimental Animal Center of Hubei province, China and were used in the present study. The animals were housed under normal laboratory conditions at 22°C under a 12-h dark/light cycle and were given food and water *ad libitum*. The rats received humane care, and the experiment strictly abided by the ethical guidelines and approval of the Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology.

SGES consisted of a long pulse (300 msec, 4 mA) and 5 short pulses (0.33 msec, 4 mA, 100 Hz). SGES was applied to the vagotomized rats while in a fasting state. The rats subjected to early subdiaphragmatic vagotomy (ESDV group) were divided into 2 groups: one group was treated with short-term SGES (ESDV + SSGES group) for 30 min/day over a period of 2 weeks, and the other group was a sham SGES (ESDV group) group containing mice that were treated for 30 min/day over a period of 2 weeks. Similarly, the rats subjected to terminal subdiaphragmatic vagotomy (TSDV) were divided into 2 groups: the long-term SGES (TSDV + LSGES) group was treated for 30 min/day over a period of 4 weeks and the sham SGES (TSDV) group was a time-matched control. The control group was treated for laparotomy alone without vagotomy or SGES. The animals underwent subdiaphragmatic vagotomy. After 24 h of fasting, the subdiaphragmatic esophagus was exposed, and ventral and dorsal truncal vagotomy was carried out. Approximately 1 cm of the bilateral vagus innervating the stomach was cut off to prevent nerves regeneration. A pair of stimulating electrodes (United States Surgical, a division of Tyco Healthcare Group LP) was implanted in the middle of the greater curvature of the stomach. After allowing 7 days for recovery, the experimental rats were subjected to electrical stimulation. The rats were sacrificed at 2 and 6 weeks, and specimens of the antrum were carefully collected. Each specimen was cut into several parts. One part was kept at -80°C for use in western blot analysis and RT-qPCR, and a second part was placed in 4% paraformaldehyde for immunofluorescence staining and terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL) assay.

RNA extraction, cDNA synthesis and RT-qPCR. The total RNA of the antrum was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and First-Strand cDNA was synthesized using PrimeScript™ RT Master Mix (Perfect Real-Time) (Takara, Otsu, Japan) according to the instructions of the manufacturer. Glyceraldehyde 3-phosphate dehydrogenase

(GAPDH) served as an internal control. The primer sequences used were as follows: connexin 43 (Cx43) forward, 5'-AGGAG TTCCACCAACTTTGGC-3' and reverse, 5'-TGGAGTAGG CTTGGACCTTGTC-3'; GDNF forward, 5'-AATGTCAC TACTTGGGTTTGG-3' and reverse, 5'-CGTTTAGCGGAAT GCTTTCTTA-3'; PGP9.5 forward, 5'-TGGAGATTAACCC GAGATGC-3' and reverse, 5'-GAGTTCCCGATGGTCTG CTTC-3'; pan-Akt forward, 5'-ATCGTGTGGCAAGATGT GTATGA-3' and reverse, 5'-CAAAATACCTGGTGTCCG TCTCA-3'; GAPDH forward, 5'-GTATGACTCTACCCACGG CAAGT-3' and reverse, 5'-TTCCCGTTGATGACCAGCTT-3'. RT-PCR was performed using the SYBR-Green PCR master mix. All reactions were performed in duplicate in a 10- μ l volume containing 1 μ l cDNA, 5 μ l SYBR-Green reaction mix (Qiagen, Hilden, Germany), 0.5 μ l sense primer, 0.5 μ l antisense primer (both from Invitrogen), and 3 μ l ddH₂O. The reaction conditions used were 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. All reactions were performed using an ABI-StepOne Real-Time system (Applied Biosystems, Carlsbad, CA, USA). Relative changes in gene expression were confirmed using the $2^{-\Delta\Delta C_q}$ method.

Western blot analysis. Fresh-frozen antrum samples were homogenized by mechanical crushing in radioimmunoprecipitation assay (RIPA) buffer containing protease inhibitor. Following incubation on ice for 30 min, the mixture was centrifuged at 12,000 rpm for 10 min at 4°C, and the supernatants used as the total protein fraction. Protein concentrations were determined using a bicinchoninic acid (BCA) protein assay kit.

The supernatants were diluted with loading buffer and the proteins were denatured at 95°C for 10 min. Lysates equivalent to 60 μ g of protein lysates were then resolved using 8–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. Non-specific binding areas of the membranes were blocked with 5% non-fat dry milk or 5% bovine serum albumin (BSA) in Tris-buffered saline containing 0.1% Tween-20 (TBST) for 1 h. Subsequently, the membranes were incubated overnight at 4°C with antibodies against GDNF (1:300; cat. no. ab18956; Abcam, Cambridge, UK), PGP9.5 (1:10,000; cat. no. NB110-58869; NovusBio, Littleton, CO, USA), p-Akt (1:1,000; cat. no. 4060), pan-Akt (1:1,000; cat. no. 13038) (both from Cell Signaling Technology, Danvers, MA, USA), Cx43 (1:1,000; cat. no. A2163; ABclonal Technology, Hubei, China) and GAPDH (1:5,000; cat. no. A01020; Abbkine, Inc., Redlands, CA, USA). After washing 3 times in TBST, the membranes were incubated with HRP-linked secondary antibody [HRP-labeled goat anti-rabbit IgG (1:5,000; cat. no. ANT020; AntGene Biotech Co., Ltd., Wuhan, China), HRP-labeled goat anti-mouse IgG (1:5,000; cat. no. ANT019; AntGene Biotech Co., Ltd.) and HRP-labeled goat anti-rabbit IgG (1:5,000; cat. no. GGHL-5P; Immunology Consultants Laboratory, Inc., Portland, OR, USA)] for 60 min at room temperature. After 3 further washes, the protein bands were detected using an enhanced chemiluminescence agent (ECL reagents). Densitometry analysis was performed using ImageJ software (Media Cybernetics, Inc., Rockville, MD, USA).

Immunofluorescence staining. The antrum tissue was labeled to determine the expression of GDNF, GFAP and PGP9.5. The

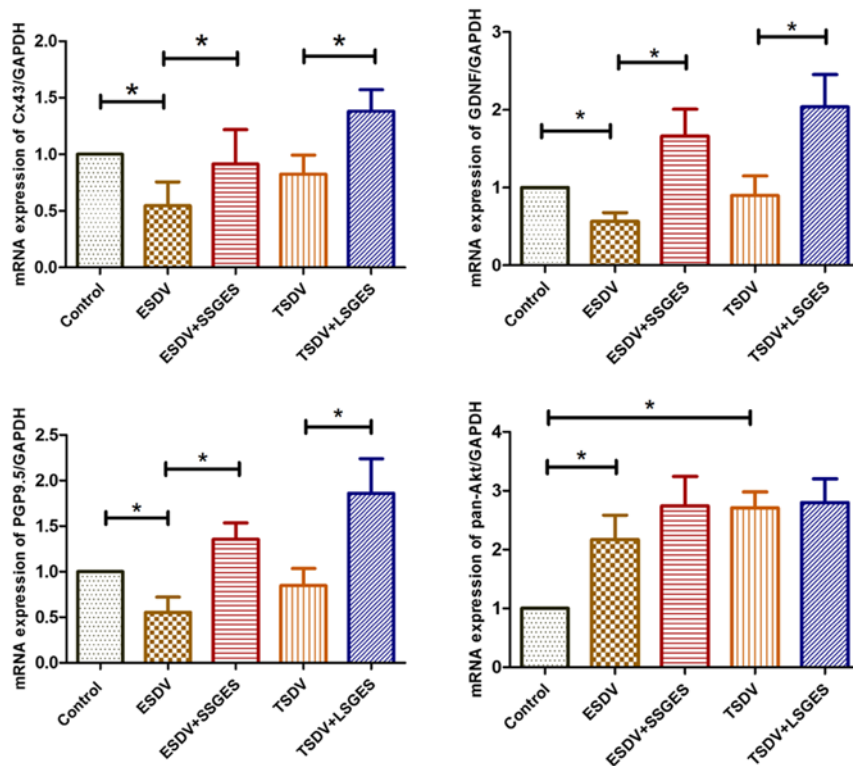


Figure 1. Connexin 43 (Cx43), glial cell line-derived neurotrophic factor (GDNF), PGP9.5, and pan-Akt mRNA expression levels in the antrum. Cx43, GDNF and PGP9.5 mRNA expression levels in the early vagotomized group were markedly decreased compared with those in the control group. Moreover, mRNA expression levels were higher in both the short-term synchronized dual pulse gastric electrical stimulation (SSGES) and long-term synchronized dual pulse gastric electrical stimulation (LSGES) groups than in the matched vagotomized groups. However, no significant difference was observed between the SGES groups and the vagotomized groups in terms of pan-Akt gene expression. * $p < 0.05$. ESDV, early subdiaphragmatic vagotomy; TSDV, terminal subdiaphragmatic vagotomy.

harvested specimens were immersed in 4% paraformaldehyde for 24 h, embedded in paraffin in a vacuum, and sliced into 5- μ m-thick sections. The sections were then dewaxed and hydrated in xylene and ethanol solutions. After boiling for 2 min for antigen retrieval, the tissues were incubated with 5% BSA for 30 min at room temperature, followed by incubation with primary antibodies to GDNF (1:50), GFAP (1:300) (both from Abcam), and PGP9.5 (1:300; NovusBio) overnight at 4°C. The sections were rinsed in phosphate-buffered saline (PBS) 3 times the following day and then incubated with secondary antibodies [Alexa Fluor 488-Donkey Anti-Rabbit IgG (1:100; cat. no. ANT024), Alexa Fluor 594-Donkey Anti-Goat IgG (1:100; cat. no. ANT031) and Alexa Fluor 488-Donkey Anti-Mouse IgG (1:200; cat. no. ANT029) (all from AntGene Biotech Co., Ltd.)], in the dark for 90 min at room temperature. Subsequently, the sections were treated with 4',6-diamidino-2-phenylindole (DAPI; 1:1500) for 10 min washed in PBS 3 times, and then sealed with a fluorescence quenching agent. The immunolabeled tissues were observed using a confocal laser scanning microscope (Nikon, Tokyo, Japan).

Apoptosis was detected with TUNEL labeling according to the manufacturer's instructions (Roche, Mannheim, Germany). Briefly, the sections were dewaxed, rehydrated and incubated with TUNEL reaction buffer (labeling solution: enzyme solution, 9:1) under dark and humid conditions. The sections were then washed to remove unbound fluorescein-dUTP and incubated with DAPI to counterstain the nuclei. Finally, the specimens were observed using a confocal laser scanning microscope (Nikon); 5 random fields were captured in each section.

Statistical analysis. All data are expressed as the means \pm SEM, and one-way ANOVA was performed to assess the difference between the control group and the vagotomized rat subgroups, followed by LSD or Dunnett's T3 analysis. A p -value < 0.05 was considered to indicate a statistically significant difference. SPSS version 17 software was used for all statistical analyses.

Results

mRNA levels of Cx43, GDNF, pan-Akt and PGP9.5. As shown in Fig. 1, the mRNA levels of Cx43, GDNF, pan-Akt, and PGP9.5 in the antrum tissue were assessed. The result of RT-qPCR revealed that the levels of Cx43, GDNF and PGP9.5 were markedly decreased in the early vagotomized group (ESDV group) compared with the control group (all $p < 0.05$). Conversely, both short-term SGES (SSGES) and long-term SGES (LSGES) significantly enhanced the expression levels of Cx43, GDNF and PGP9.5 compared with the matched vagotomized groups (all $p < 0.05$). The expression of pan-Akt in the vagotomized groups was markedly upregulated compared with the control group (both $p < 0.05$), whereas there was no difference between the vagotomized groups and the SGES groups (both $p = 1.00$).

Protein levels detected by western blot analysis. The protein levels of Cx43, GDNF, p-Akt, pan-Akt and PGP9.5 are depicted in Fig. 2. The expression levels of Cx43, GDNF and PGP9.5 in the early vagotomized group were markedly lower than those in the control group (0.51325 ± 0.06376 vs. 1.33943 ± 0.04699 ,

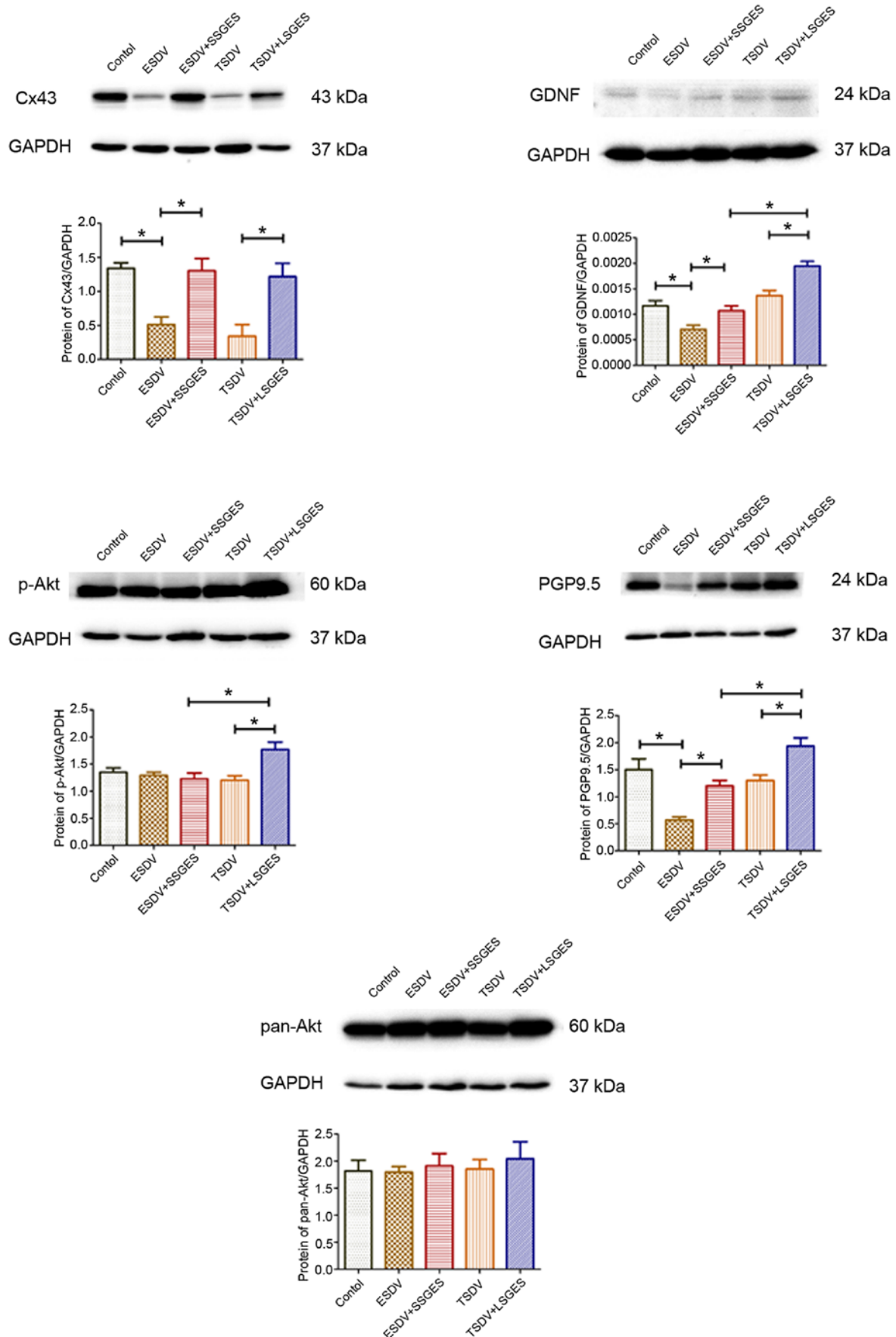


Figure 2. Connexin 43 (Cx43), glial cell line-derived neurotrophic factor (GDNF), p-Akt, pan-Akt and PGP9.5 protein expression levels in the antrum as measured by western blot analysis. Cx43, GDNF and PGP9.5 protein expression levels were significantly decreased in the early vagotomized group compared with the control group, whereas short-term synchronized dual pulse gastric electrical stimulation (SSGES) upregulated protein expression compared with that in the early vagotomized group. p-Akt protein expression was markedly increased in the long-term synchronized dual pulse gastric electrical stimulation (LSGES) group compared with the terminal vagotomized group. There were no significant differences in pan-Akt protein levels among the different groups. * $p < 0.05$.

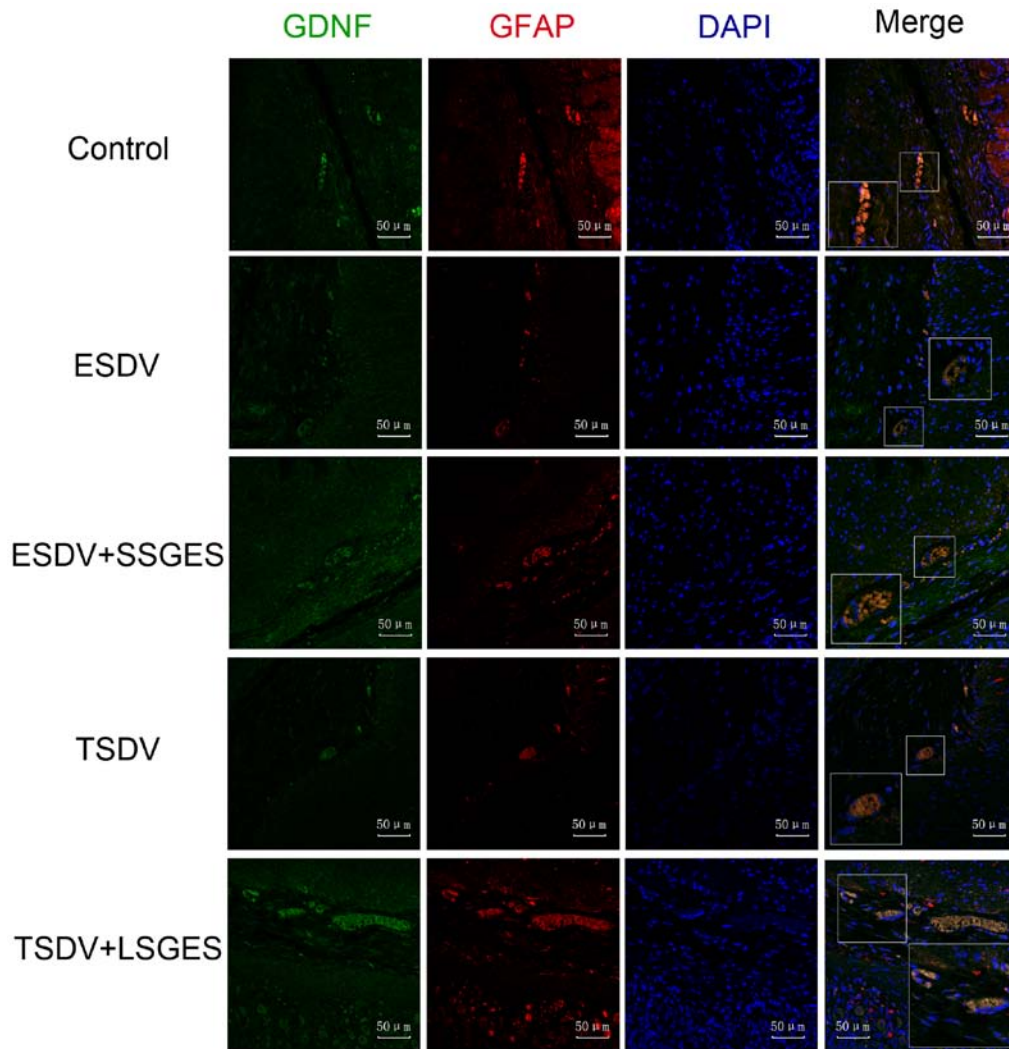


Figure 3. Confocal images of enteric glial cells labeled with glial cell line-derived neurotrophic factor (GDNF) (green) and GFAP (red). GDNF and GFAP protein expression levels were lower in the vagotomized groups than in the control group. short-term synchronized dual pulse gastric electrical stimulation (SSGES) or long-term synchronized dual pulse gastric electrical stimulation (LSGES) increased the expression of GDNF and GFAP.

$p=0.004$; 0.00070 ± 0.00005 vs. 0.00117 ± 0.00020 , $p=0.022$; 0.56762 ± 0.03344 vs. 1.50193 ± 0.11532 , $p=0.043$). Short-term SGES obviously upregulated the levels of these proteins compared with the corresponding age-matched vagotomized groups (1.30507 ± 0.10339 vs. 0.51325 ± 0.06376 , $p=0.028$; 0.00107 ± 0.00005 vs. 0.00070 ± 0.00005 , $p=0.045$; 1.20183 ± 0.05780 vs. 0.56762 ± 0.03344 , $p=0.01$). Long-term SGES significantly increased the expression of p-Akt compared with the terminal vagotomized group (1.76987 ± 0.07826 vs. 1.20364 ± 0.04708 , $p=0.034$). However, no significant difference in pan-Akt expression was observed between the vagotomized groups and the SGES groups (all $p>0.05$).

Immunofluorescence labeling of GDNF, GFAP and PGP9.5.

The immunofluorescence staining of GDNF, GFAP and PGP9.5 is shown in Figs. 3 and 4. As shown in Fig. 3, GDNF and GFAP co-expression in the vagotomized groups was markedly lower than that in the control group in the myenteric plexus (all $p<0.05$). Conversely, long and short-term SGES significantly increased the expression levels of GDNF and GFAP compared with the levels in the vagotomized groups (all

$p<0.05$). Consistently, the change in PGP9.5 expression was similar to that observed for GDNF and GFAP (Fig. 4).

Assessment of cell apoptosis by TUNEL assay. As shown in Fig. 5, few TUNEL-positive cells were observed in the control group. However, more positive cells were found in the early and terminal vagotomized groups compared with the control group ($p<0.05$). In addition, short- and long-term SGES markedly decreased the number of TUNEL-positive cells compared with those in the matched vagotomized groups ($p<0.05$).

Discussion

GES has been applied to alleviate gastrointestinal symptoms and promote gastrointestinal motility; however, the mechanisms involved in the effects of SGES remain elusive. In the present study, we found that SGES enhanced the release of GDNF following the upregulation of its downstream pathway, PI3K/Akt, and the inhibition of enteric neuronal loss in the antrum tissue.

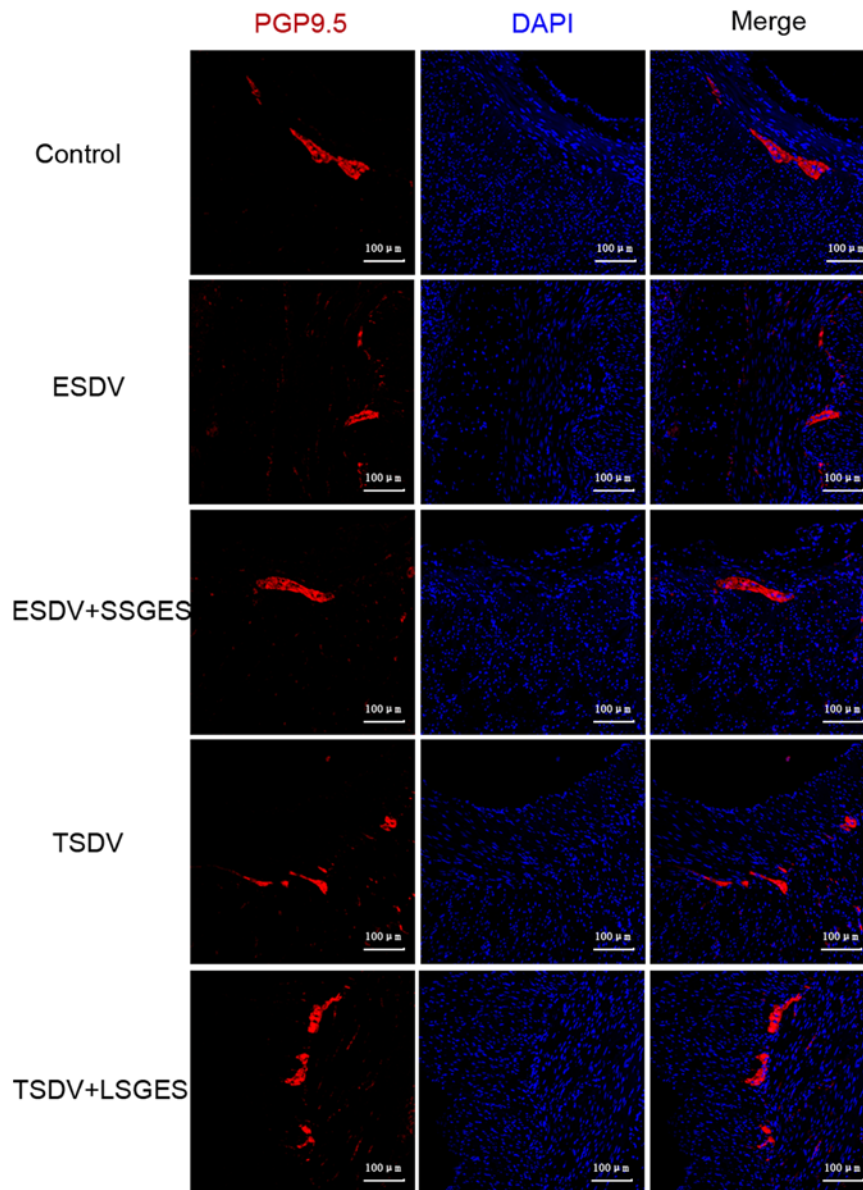


Figure 4. PGP9.5 protein expression in the antrum as measured by immunofluorescence assay. PGP9.5 protein expression was lower in the vagotomized groups than in the synchronized dual-pulse gastric electrical stimulation (SGES) groups. The change was similar as that for glial cell line-derived neurotrophic factor (GDNF) protein expression. Scale bar, 50 μ m.

The severity of enteric neuronal loss parallels gastrointestinal motility defects in zebrafish (13). Gastric and small intestinal motility damage is related to a decrease in the number of neuronal nitric oxide synthase (nNOS)-immunoreactive myenteric neurons following chronic alcohol consumption (14). In rats with streptozotocin (STZ)-induced diabetes, the change in aquaporin 1 immunoreactive neurons may lead to gastrointestinal dysfunction (15). Moreover, the survival and neurogenesis of transplanted neural stem cells in the adult gut are key issues in the use of stem cell therapy for treating gastrointestinal motility disorders (16). In this study, we found that the expression of PGP9.5, an enteric neuronal marker, was significantly decreased, and more TUNEL-positive cells were found in the vagotomized rats. These results likely indicate injury to or the loss of enteric neurons following vagotomy and may partly explain the role of gastrointestinal dysmotility in enteric neuropathies.

GDNF is located in glia or Schwann cells in the human gut (17). GDNF and neurturin play prominent roles in the survival and proliferation of enteric neurons and glial progenitors *in vitro* (18); the ENS completely fails to mature in mice lacking GDNF (19). Abundant GDNF synthesis has been found in enterocytes, promoting wound healing and barrier maturation (20). Disorders of gastrointestinal motility have been associated with signaling components of GDNF in enteric neuropathies (21). In addition, GDNF contributes to synaptophysin and synaptobrevin expression and formatted neuronal varicosities, which induces functional neuronal networks (22). A previous study indicated that GDNF and brain-derived neurotrophic factor expression levels are upregulated and that cellular apoptosis is attenuated by acupuncture in the hippocampi of rats experiencing hypoxia-ischemia (23). Furthermore, GDNF has a neurotrophic effect on diabetes-induced neuronal apoptosis in the hippocampus (24) and

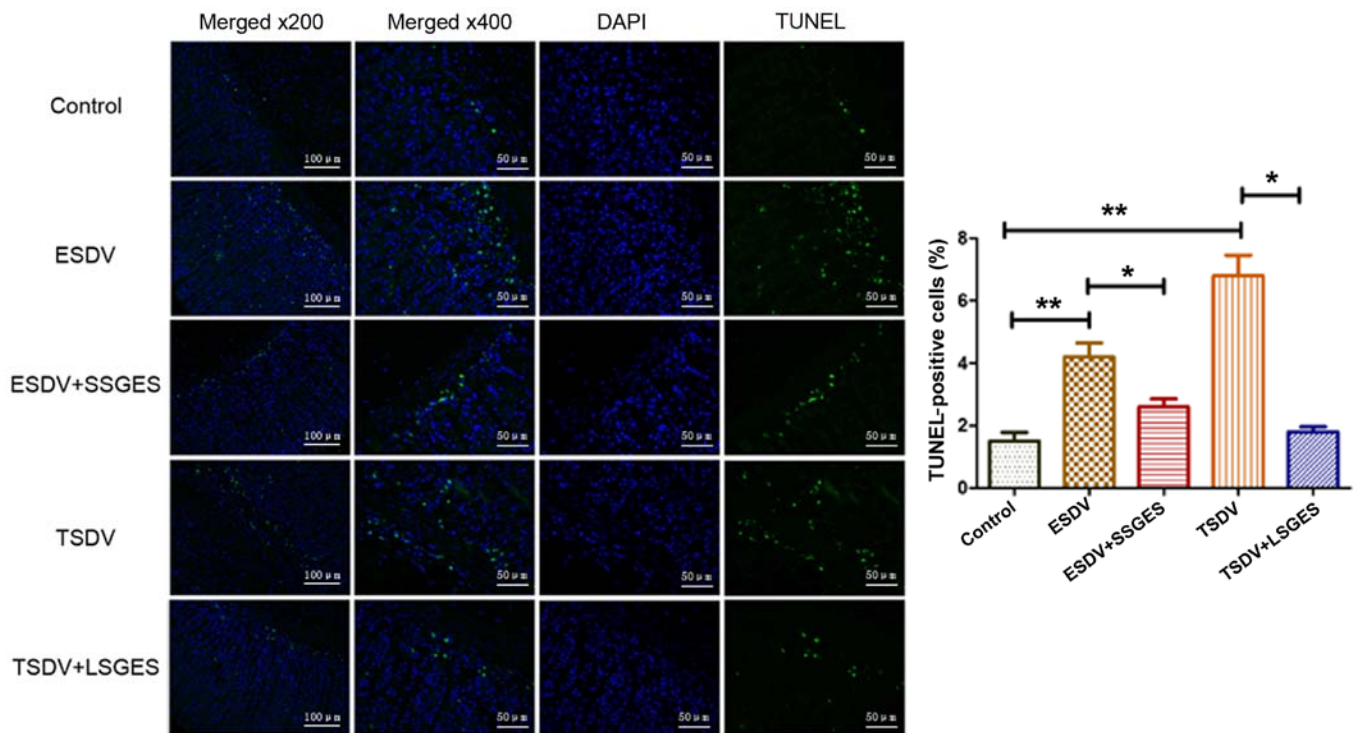


Figure 5. Apoptosis in the antrum as measured by terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL) staining. Few TUNEL-positive cells were observed in the control group. The number of TUNEL-positive cells was markedly higher in the vagotomized groups than in the synchronized dual-pulse gastric electrical stimulation (SGES) groups. * $p<0.05$ and ** $p<0.01$.

enhances the migration of colon cancer cells (25) via the PI3K/Akt pathway.

In the present study, we demonstrated that GDNF expression was markedly decreased in the stomachs of rats subjected to early vagotomy, and SGES upregulated GDNF and p-Akt expression. Additionally, no change was observed in the pan-Akt levels in either the vagotomized groups or the SGES groups. These data indicate that GDNF and p-Akt probably play a role in the damage to enteric neurons, and that SGES likely protected the loss of enteric neurons via GDNF and the PI3K/Akt pathway.

In recent years, EGCs have been increasingly regarded as crucial partners of enteric neurons in the regulation of gastrointestinal function and structure. Enteric glia-neuron communication is beneficial to neuronal maintenance, survival and function. As a result, evidence indicates that enteric glial cells release neurotransmitters, such as reduced glutathione and 15d-PGJ2, which exert neuroprotective effects (26,27). Enteric glial ablation or disruption is involved in the dysfunction of enteric neurons that contributes to intestinal motility disorders (28,29). Indeed, the present study demonstrated that GFAP expression, specifically its expression in EGCs in the antrum tissue, was decreased and followed almost the same trend as PGP9.5 in the vagotomized groups. We speculate that the injured EGCs contributed to neuronal damage as a dense network of EGCs was packed around the enteric neurons.

GES is used in the treatment of gastrointestinal dysfunction including nausea, vomiting, early satiety, abdominal bloating and loss of appetite. GES has been shown to alleviate symptoms, such as nausea, loss of appetite and early satiety, with

optimal responses observed in 75% of patients with refractory gastroparesis (11). The effect of GES may be involved in central, neuronal and hormonal mechanisms that GES activates in gastric distension-sensitive neurons and in the improved expression of cholecystokinin in the hippocampus (30). GES applied with the appropriate parameters has become a promising treatment for gastrointestinal functional disorders. In the present study, we demonstrated that SGES with a long pulse (300 msec, 4 mA) followed by 5 short pulses (0.3 msec, 100 Hz, 4 mA) markedly upregulated GDNF expression together with p-Akt and PGP9.5, and reduced the number of TUNEL-positive cells. Our previous studies demonstrated that SGES activated EGCs and improved gastric emptying (31,32). This result indicates that SGES restores injured ENS and improves gastric motility.

Intriguingly, a previous study demonstrated that hepatic electrical stimulation decreased fasting and fed blood glucose in normal and diabetic rats, a result that may be mediated by the release of glucagon-like peptide-1 (GLP-1) (33). Moreover, another previous study demonstrated that SGES using optically appropriate parameters improved vagotomy-induced impairment in gastric accommodation resulting from vagotomy (34). It has also been shown that SGES improves gastric motility, an effect that may be related to the activation of enteric glial cells (31).

Cx43 belongs to the connexin family of gap junction proteins and is widespread in the body. Cx43, which is limited to enteric glia in the myenteric plexus of the mouse colon, directly mediates Ca^{2+} responses and indirectly contributes to whole-gut transit (35). In addition, Cx43 serves as a major gap junction protein that deteriorates in inflamed white matter in

multiple sclerosis (36). Our data indicated that Cx43 expression was markedly reduced in the vagotomized groups and that short- or long-term SGES increased Cx43 expression. Our previous study found that SGES improved gastric emptying in vagotomized rats through the activation of enteric glial cells (32). More research is required to determine whether SGES enhances the cross-talk between enteric glial cells via the Cx43 protein and further promotes the release of GDNF.

GES is used in the treatment of certain diseases. Numerous studies have shown that the use of GES with appropriate parameters is an effective method for the treatment of refractory gastroparesis resulting from gastric neuropathy (37-39). Of note, GES contributes to significant weight loss as the result of changes in eating behavior (40) and the activation neurons in the hypothalamus (41). Neurodegenerative diseases of the central nervous system and injury to the central nervous system lead to delayed gastric emptying. Gastric emptying is delayed in Parkinson's disease, probably as the result of neurodegeneration in the dorsal motor nucleus of the vagus nerve (42,43). Delayed gastric emptying occurs in patients with traumatic brain injury, and treatment with prokinetic drug is needed to relieve enteral feeding tolerance (44). Spinal cord injury can lead to damage to gastric motility and emptying (45). Collectively, these findings indicate that GES has therapeutic potential for treating gastrointestinal dysfunction due to ENS or in central nervous system.

In conclusion, in this study, we demonstrated that GDNF, p-Akt and PGP9.5 expression was decreased and more TUNEL-positive cells were observed in the vagotomized rats. These results imply that GDNF and the PI3K/Akt signaling pathway play vital roles in neuropathy of the gastrointestinal tract. Moreover, SGES upregulated the expression of GDNF, p-Akt and PGP9.5 in the vagotomized rats and inhibited the apoptosis of enteric neurons. This finding suggests that SGES may be a promising treatment for enteric neuropathies and functional gastrointestinal disorders.

Acknowledgements

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