Effectiveness of Electroacupuncture at Zusanli (ST36) on the Immunohistochemical Density of Enteroendocrine Cells Related to Gastrointestinal Function

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Abstract
The purpose of this study was to examine the effects of electroacupuncture at Zusanli on the immunohistochemical density of enteroendocrine cells related to gastrointestinal function. The authors investigated the histochemical changes of mucous substances and immunohistochemical density of gastrin, serotonin, calcitonin gene-related peptide (CGRP), insulin, and pancreatic polypeptide (PP) secreting cells in rats. Staining density of mucous substances and the enteroendocrine cells of the gastrointestinal tract was observed with histochemical and immunohistochemical methods. Stainless steel needles with a diameter of 0.25 mm were inserted into Zusanli (St36, 5 mm below the head of the fibula under the knee joint, and 2 mm lateral to the anterior tubercle of the tibia) and connected to an electrical stimulator. The electroacupuncture (EA) stimulation was delivered for 30 minutes at 10 mA, 2 Hz in EA stimulation (2EA group) or 4 Hz in EA stimulation (4EA group) in each experimental group. In 4EA stimulation at the Zusanli, staining density of Alcian blue-periodic acid-Schiff on mucous substances of the stomach body was stronger than those of the 2EA and control groups. Periodic acidi-Schiff staining
1. Introduction

It has already been shown that the approach of Oriental medicine to treat disease is clinically effective. Thus, medicine (herbs), acupuncture and moxibustion, and physical therapy are applied together. Acupuncture therapy improves physical function and pathologic symptoms by medicine and stimulation of acupuncture on Zusanli using meridian theory to recover normal physical activities and treat disease. Recently, various new acupuncture therapies other than traditional methods by manual acupuncture have been developed for clinical use [1,2].

The electroacupuncture (EA) method is a new technique that strengthens stimulation using an electric charge and replaces the magnetic needle based on meridian and acupuncture points in Oriental medicine, and is known for its effect on analgesia [3], the cardiovascular system [4], the digestive system [5], and the nervous system [6].

Studies on EA related to the gastrointestinal (GI) tract, especially on the relationship between "Hegu" (LI4) or "Sanjinyajiao" (SP9) and intestinal motility, the relationship between stimulation of Zusanli and "Yinlingquan" (GB34) and gastrin level, and therapeutic effect in an acute pancreatitis model, have been published [7–10]. EA has been more effective in the treatment of functional disease, such as neuralgia or paralysis, because of its continuous stimulation and objective control of the degree of stimulation. EA exhibits a different therapeutic effect on stimulation condition. There is a difference in the activity on c-fos protein in neurons and serotoninergic neurons depending on various patterns of frequency, voltages, and wave patterns and applying hours of electric current for EA stimulation [11,12]. Lee et al. [13] suggested that if EA is stimulated to the Neiguan (Pe6 g), which is a useful meridian point for all visceral injuries and neuropsychiatric diseases, change in δ-wave affects the thalamus and cerebral cortex and in turn causes a change in the amount of endocrine secretion. Also, the δ-wave directly and indirectly has a relationship with secretion of the growth hormone-releasing hormone interleukin-1, growth hormone, cholecystokinin, leptin, somatostatin, and insulin growth factor-1 in humans. Thus, EA shows clinical effect by controlling the nervous system and endocrine system.

In Oriental medicine, it has been known that the stimulation of acupoints directly affects relevant meridian points and visceral organs that achieve the effect of acupuncture therapy. Recently, developments in neuroscience have proved that various diseases are influenced by the brain. In addition, studies on brainwave change by imaging technologies such as positron emission tomography and functional magnetic resonance imaging or acupuncture stimulation, and the use of pseudorabies virus, which is a transsynaptic virus, as a neural tracer handle metabolic activities [14] and a change of brainwave [13] that appears in the central nervous system after EA stimulation or the relationship between visceral organs and the autonomic nerve center [15]. Thus, the recent researches on the relationship between acupoints and the cerebral cortex, and the functions of the brain with a relationship to the acupoints and visceral organs, have been highlighted.

This study is focused on the morphologic observation of the mutual relationship between EA stimulation of Zusanli and the function of the GI tract and neurotransmitter in the central nervous system. After Zusanli, which is usually used to control functions and disease of the GI tract, is stimulated by EA, immunohistochemical density of serotonin and calcitonin gene-related peptide (CGRP)-secreting cells that exist in the central nervous system and the change in density of immunostaining of various enteroendocrine cells existing in the GI tract is observed.

2. Materials and method

2.1. Experimental animals

Twenty adult male Sprague-Dawley rats weighing 200 to 250 g were used in this study. Rats were treated in accordance with standard guidelines for laboratory animal care and according to the regulations of the experimental animal ethics committee of Woosuk University, Korea. Animals were randomly divided into a control group and experimental groups (2EA group and 4EA group). Each group comprised six animals. After induction of anesthesia with 1% sodium pentobarbital (50 mg/kg, i.p.), all rats were mounted in a stereotaxic device.

2.2. EA stimulation

EA stimulation is performed on Zusanli (St36), corresponding to the point in humans by the proportional method according to Koh [16]. A Grass S88 dual channel stimulator is used for EA stimulation. A constant current unit is attached to the stimulator and either 10 mA, 2 Hz, or 4 Hz of single electrode wavy pattern is conducted on the animals in the experimental groups. For the 2EA and 4EA experimental groups, acupuncture is treated on left and right Zusanli, stimulate + and - electrodes to the relevant acupoint and changed electrodes every 5 minutes to prevent adaptation on the stimulation. Twice as much strength of stimulus where the first muscle cramp appears in the experimental animals is used. Total stimulation time
is 30 minutes. During the entire experimental period that EA stimulation is treated, experimental animals are anesthetized with 1% sodium pentobarbital so that stress during the EA stimulation period is minimized. The level of anesthesia reflects that they are under sedation that breathing rate and temperature are maintained. The control group is used to verify the influence of anesthesia used for the experiment on the neural activity of experimental animals: first, use the same level of anesthesia; second, treat acupuncture on the relevant acupoints; third, connect electric line but stay for 30 minutes, not applying electric stimulus; fourth, perform immunohistochemistry on the tissues of the central nervous system and the GI tract.

2.3. Tissue preparation

Experimental animals that underwent EA are given an anesthetic with chloroform after 1 hour, and undergo perfusion via the left cardiac ventricle with approximately 250 to 300 mL of 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate-buffered (PB) solution after perfusion with 100 to 150 mL of 0.9% saline. The GI tract and spinal cord were quickly removed after perfusion and administered the same fixative for 12 hr at 4 °C. Fixed spinal cord segments (T9-10 and L4-5) were rinsed for 24 hours in 20% sucrose and sectioned serially at 40-μm thickness in a cryostat (Leica, Wetzlar, Germany) at -20 °C. Each section was then mounted on gelatin-coated slides and stored at -70 °C until use. The fixed GI tract (pylorus and body of the stomach, proximal duodenum, pancreas, and distal colon) were rinsed for 48 hours in 70% alcohol and then embedded in paraffin. Seven-μm transverse sections were cut on a microtome (Reichert-Jung Histocut 820, Wetzlar, Germany), stained by hematoxylin and eosin (H & E) and Alcian blue-periodic acid-Schiff reagent (AB-PAS), and immunohistochemical staining methods.

2.4. H & E and AB-PAS stains

For histologic staining, paraffin was removed in xylene and the sections were rehydrated with graded ethanol. H & E staining was performed according to general protocol. AB-PAS staining for special stain was performed to observe the change of mucous substances in the pylorus and the body of the stomach [17]. After washing in distilled water, the sections were then stained with Alcian blue (AB) solution for 20 minutes at room temperature. After washing for 2 minutes, the sections were oxidized for 10 minutes in 0.5% periodic acid solution and washed for 5 minutes. Then, the sections are stained in periodic acid-Schiff (PAS) solution for 10 minutes and washed in sulfurous solution for 2 minutes. After the dehydration and clearing process, the sections are permounted and the staining density of mucous substances observed through an optical microscope.

2.5. Immunohistochemical methods

In this experiment, the avidin-biotin peroxidase method is used for immunohistochemistry [18]. Immunostaining of the spinal cord sections were performed on both the free-floating sections and the gelatin-coated slides. The GI tract sections were rehydrated and immunostained with primary antibodies against gastrin (1:300, DAKO, Carpenteria, CA, USA), serotonin (1:5,000, Sigma-Aldrich, St. Louis, MO, USA) and CGRP (1:8,000, Sigma-Aldrich, St. Louis, MO, USA), insulin (1:200,000, Sigma-Aldrich, St. Louis, MO, USA) and pancreatic polypeptide (PP, 1:300, DAKO, Carpinteria, CA, USA) followed by an appropriate biotinylated secondary antibody. Stains were visualized using the ABC kit (Vector, Burlingame, CA, USA), reacted with diaminobenzidine (DAB; Sigma-Aldrich, St. Louis, MO, USA) as a substrate, and counterstained with hematoxylin. Images were observed and captured on an Axioscope (Carl Zeiss, Göttingen, Germany) microscope equipped with a digital camera (Nikon, Japan). All incubation steps were performed in a humidified chamber.

3. Results

3.1. Histochemical staining reactions of Gastric Mucosa

Acupuncture is applied to Zusanli of all groups. The EA stimulation was delivered for 30 minutes at 10 mA, 2 Hz in EA stimulation (2EA group), or 4 Hz in EA stimulation (4EA group) in each experimental group, and voltage is not stimulated in the control group. The histochemical change of mucous substances in the body of the stomach is observed by AB-PAS staining. The results indicate that in the control group, mucous substances of surface epithelium adjacent to the lumen have a weak PAS staining reaction and a strong staining reaction to AB (Fig. 1A). In the 2EA group, mucous substances of surface epithelium to PAS staining reaction and AB staining in the gastric pit indicate aspects similar to that of the control group (Fig. 1B). However, in the 4EA group, the PAS staining reaction of mucous substances of surface epithelium is slightly stronger than that of the control group and 2EA group (Fig. 1C). Especially in the 4EA group, many cells that stained strong above the surface epithelium is stronger than that of the control group and 2EA group. The histochemical change of mucous membrane of the pylorus of the stomach is observed by AB-PAS staining. The results indicate that PAS staining of surface epithelium adjacent to mucous lumen and epithelium in the gastric pit below the surface epithelium is stronger than that of the control group and 2EA group, and there is no significant difference in AB staining among the three groups. From these results, experimental groups that stimulate EA (2EA group and 4EA group) promote activity of neutral mucin-secreting cells in the 4EA group rather than the control group.

3.2. Immunohistochemical staining reaction in the enteroendocrine cells of Gastric Mucosa

After stimulating Zusanli with EA, the immunohistochemical staining reaction of gastrin-secreting cells in the pylorus of the stomach is observed. The results indicate that gastrin-secreting cells are mostly distributed to the...
glandular portion in the base of the mucous membrane. Immunostaining reaction is strongest in the 2EA group (Fig. 2B), intermediate in the 4EA group (Fig. 2C), and the weakest in the control group (Fig. 2A). Thus, groups stimulated by EA (2EA and 4EA groups) promoted activity of gastrin-secreting cells more than the control group.

After stimulating Zusanli with EA, immunohistochemical staining reaction of serotonin-secreting cells in the mucous membrane of the pylorus of the stomach is observed. The results indicate that serotonin-secreting cells are mostly distributed to the glandular portion, which is a basal part of the mucous membrane of the pylorus of the stomach. Immunostaining reaction indicates that it is strongest in the 2EA group (Fig. 3B), intermediate in the 4EA group (Fig. 3C), and weakest in the control group (Fig. 3A). The groups stimulated by EA (2EA and 4EA groups) promote the activity of serotonin-secreting cells rather than the control group.

After stimulating Zusanli with EA, immunohistochemical staining reaction of CGRP-secreting cells in the mucous membrane of the pylorus of the stomach is observed. The results indicate that CGRP-secreting cells are mostly distributed to the glandular portion, which is the basal part of mucosa of the pylorus of stomach. Immunostaining reaction indicates that it is strongest in the 2EA group (Fig. 4B), intermediate in the 4EA group (Fig. 4C), and weakest in the control group (Fig. 4A). The groups stimulated by EA (2EA and 4EA groups) promote the activity of CGRP-secreting cells rather than the control group.

After stimulating Zusanli with EA, the results of immunohistochemical staining reaction of insulin-secreting cells in pancreatic islets indicate that insulin-secreting cells are scattered in the pancreatic islets and the staining reaction is stronger in the 2EA (Fig. 5B) and 4EA (Fig. 5C) groups than in the control group (Fig. 5A), which shows the weakest immunohistochemical reaction. The 2EA and 4EA groups promote activity of insulin-secreting cells more than the control group.

Figure 1 Photomicrographs (× 100) of the stomach body showing surface epithelium and gastric pits stained by AB-PAS. Density of surface mucous epithelium of the 4EA group was stronger than that of the control and 2EA groups. (A) Control group. (B) 2EA group. (C) 4EA group. Arrow, periodic acid-Schiff positive neutral mucin; arrowhead, Alcian blue positive acid mucin. AB-PAS = Alcian blue-periodic acid-Schiff stain; EA = electroacupuncture.

3.3. Immunohistochemical staining reaction of neurotransmitters in the spinal cord

After stimulating Zusanli with EA, the results of immunohistochemical staining reaction of CGRP are observed in T9-10 spinal cord sections. The results indicate that the immunostaining reaction to CGRP is stronger in the 2EA (Fig. 7B) and 4EA groups (Fig. 7C) than the control group (Fig. 7A) at the mediolateral part of lamellae I, II, and III. In L4-5 spinal cord sections, the immunostaining reaction to CGRP is stronger in the 2EA (Fig. 8B) and 4EA groups (Fig. 8C) than in the control group (Fig. 8A) at the mediolateral part of lamellae I, II, and III.

4. Discussion

EA is a therapy that combines mechanical stimulus and electric stimulus through current on a needle pole, and is known to have an effect on analgesia [3], the

Figure 2 Photomicrographs (× 100) of stomach pylorus stained by the immunohistochemical method. Density of gastrin immunoreactive cells in the 2EA group was stronger than that of the control group. The strongest staining group was the 2EA group. (A) Control group. (B) 2EA group. (C) 4EA group. Arrows, gastrin immunoreactive cells. EA = electroacupuncture.
cardiovascular system [4], the digestive system [5] and the nervous system [6]. Therapeutic effect differs depending on various patterns of frequency, voltage, wave pattern, and duration of electric current for EA stimulation, and stimulation with fast speed and overthreshold are required for effective treatment [11,12].

EA stimulus indicates analgesia and various autonomic motor responses [19]. Recent studies have reported that EA causes secretion of gastric acid [20], change in pupil diameter [21], and secretion of adrenomedullary hormone [22] via somatic afferent nerve and autonomic efferent nerve reflectively from anesthetized animals.

In the study related to EA stimulation and function of the GI tract, peristaltic movement is enhanced or reduced when stimulating Zusanli with EA and secretion of gastric acid is restricted by sensory nerves that control the stomach [23]. If Zusanli is stimulated by EA in 50 Hz, there is no change in movement of the small intestine but its capacity is significantly increased in 2 Hz and 100 Hz [24].

In this experiment, EA is applied to Zusanli that controls function and disease of the GI tract. Then, immunostaining of various enteroendocrine cells existing in the GI tract, the neurotransmitter serotonin existing in the central nervous system, and change in the expression of CGRP were observed to morphologically identify the mutual relationship between EA stimulus of Zusanli, function of the GI tract, and the neurotransmitter in the central nervous system.

Mucin, found in the mucous membrane of the GI tract, consists of heavy mucin glycoprotein, which plays an important role in the physical defense mechanism of mucous membrane of the GI tract [25]. Mucin in the GI tract is secreted from mucous cells of surface epithelium and glandular epithelium. Mucins secreted from two types of mucin cells are different in their physical function of mucous membrane of the stomach [26]. This change in the composition of mucin and biochemical characteristics plays a role in the etiology of diseases such as colitis. Also, quantitative and qualitative change in composition of carbohydrate of mucin glycoconjugate causes digestive diseases such as ulcerative colitis and colorectal cancer [27]. Mucin shows different staining reactions according to regions of the stomach-body, pylorus, and surface epithelium of mucous membrane, and glandular epithelium of the deep layer of mucous membrane. AB-PAS double staining in particular indicates acid mucin cells on deep layer of glandular epithelium turn blue with AB; neutral mucin cells on the surface epithelium turn red with PAS; mucous neck cells immediately below the surface epithelium turn dark jade green with AB and PAS simultaneously [28].

Mucin increases biosynthesis of mucin by choline neural stimulus in pylorus more than in the body of the stomach and enhances biosynthesis of mucin in the glandular epithelium in the deep layer more than surface epithelium even in the same region [29]. Therefore, secretion of mucin in the stomach is controlled by the regions (pylorus and body) of mucous membrane of the stomach, and different kinds of mucin are secreted by the location even in the same region [30].

In this experiment, the change of mucous membrane is observed by AB-PAS staining. The results indicate that in the 4EA group, PAS staining response to the mucous substances on the surface epithelium of mucous membrane is stronger than that of the control group. The strongest staining was found in the 2EA group.
of the body of the stomach is stronger than in the control group and the 2EA group. AB staining response to the epithelium at the gastric pit is also slightly stronger than in the control group and the 2EA group. In the 2EA group in particular, many cells that are strongly stained by PAS or AB are found in the surface epithelium of the body of the stomach. In the 4EA group, surface epithelium adjacent to lumen of mucosa of the pylorus of the stomach and epithelium stained by PAS in the gastric pit below the surface epithelium are stained stronger than in the control and 2EA groups. In mucosa of the duodenum, the 2EA and 4EA groups show more cells stained by PAS on the epithelium of the intestinal gland than the control group. Between the two experimental groups, the 4EA group shows more cells stained by PAS than the 2EA group. From these experimental results, it is shown that the 4EA and 2EA groups enhance activity of neutral mucous-secreting cells more than the control group. Between the two groups, the 4EA group increases activity of neutral mucin-secreting cells more than the 2EA group.

For neurotransmitters and endocrines that play an important role in movement and function of the GI tract, acetylcholine is secreted from the motor end plate by excitement of the vagus nerve, a parasympathetic nerve that controls the GI tract and pelvic nerve that is bound to the muscarinic cholinergic receptor that contracts muscles, stimulates gastrin from G cells in the pylorus of the stomach, and enhances movement of the stomach [31]. However, atropin in the muscarinic receptor and nicotine in the nicotinic receptor antagonistically react against acetylcholine and restrict the movement of the GI tract. Also, because of the excitement of sympathetic nerve, noradrenergic neuron of adrenal medulla and adrenergic neuron is bound to α-adrenergic receptor, which restricts contraction of the cholinergic fibers of smooth muscle. Epinephrine is bound to β-adrenergic receptor of neuron, which relaxes smooth muscle of the GI tract [32]. Neuro-modulation of the GI tract is controlled by chemical transmitters: acetylcholine and catecholamine as well as a number of peptides such as serotonin, substance P, vasoactive intestinal peptide, secretin, and bombesin, which are found in the neuronal cells of each region of the body and brain. Opioid, whose secretion is promoted by acupuncture, restricts peristalsis of the GI tract, causes contraction of the pylorus to delay emptying of gastric contents, and restricts secretion from the GI tract [33,34]. Gastrin among endocrine cells of the GI tract is a hormone that stimulates secretion of gastric acid, which locally participates in contraction of smooth muscle of the oxyntic region and the mucous membrane of the pyloric region. Gastrin activates histamine-storing cells, stimulates the release of histamine, and accelerates the release of gastric acid [35]. When gastric acid is secreted, for the mucous membrane of the stomach increases protective function, gastrin directly or indirectly stimulates biosynthesis of mucin to be the medium of histamine [36].

In this experiment, after stimulating Zusanli with EA, cells that release gastrin, serotonin, and CGRP from mucous membrane of the pylorus of the stomach are distributed to the glandular portion of the basal area of mucous membrane. For immunostaining reactions, the strongest immunostaining response is shown in the 2EA group, the

**Figure 5** Photomicrographs (× 200) of pancreatic islets stained by the immunohistochemical method. Density of insulin immunoreactive cells in 2A and 4EA groups were stronger than that of the control group. (A) Control group. (B) 2EA group. (C) 4EA group. Arrows, insulin immunoreactive cells. EA = electroacupuncture.

**Figure 6** Photomicrographs (× 200) of pancreatic islets stained by the immunohistochemical method. Density of pancreatic polypeptide immunoreactive cells in 2EA and 4EA groups was stronger than that of the control group. (A) Control group. (B) 2EA group. (C) 4EA group. Arrows, pancreatic polypeptide immunoreactive cells. EA = electroacupuncture.
intermediate level of immunostaining response is in the 4EA group, and the weakest immunostaining response is in the control group. It indicates that the experimental groups in which EA is applied (2EA and 4EA groups) promote activity of gastrin, serotonin, and CGRP-secreting cells more than the control group.

With regard to the change in gastric enzyme and hormone related to digestive function when acupuncture is applied, Zhou et al. [37] suggested that the level of gastrin increased when stimulating Zusanli (St 36), Pishu (UB20), and Neiguan (Pe6), and a similar result was obtained from patients with gastritis who received acupuncture treatment [38]. However, Wu et al. [39] reported that when EA was applied in the human, serum gastrin concentration decreased, indicating the opposite result. Liu et al. [40] stated that when acupuncture was applied to Zhongwan (Ren12), Neiguan (Pe6), and Zusanli (St36) for patients with duodenal ulcer, the level of G cell and gastrin decreased, whereas for patients with chronic atrophic gastritis, the level of G cell and gastrin increased, which indicates a difference according to disease. Also, Uvnas-Moberg et al. [41] reported that when stimulating with EA with 2 to 5 V, 2 Hz, blood gastrin, cholecystokinin and somatostatin dramatically increased, whereas results of the group in which vatotomy was performed or atropin was administered before the experiment indicate that gastrin and cholecystokinin were not released.

Serotonin consists of monoamine, which is widely distributed to endocrine cells of the nervous system, GI tract, and other visceral organs. In the GI tract, serotonin participates in peristalsis by restriction of secretion of gastric acid and contraction of smooth muscle [42] and shows high frequency of appearance rather than endocrine cells of other GI tracts, so that it is important to physical function of the digestive tract [43]. Solcia et al. [44] reported that serotonin played a role in regulating function of the digestive tract and intestine.

Zhou et al. [37] reported that blood 5-hydroxytryptamine (HT) decreased but the level of gastrin in G cells increased when dog philtrum was stimulated with EA. Here, given both gastrin and 5-HT restricted gastric movement, EA applied to philtrum enhanced storage of gastrin in G cells and 5-HT in enterochromaffin cells and restricted their release, which finally restrict gastric motility [37].

The results of these studies are compared to those of this experiment. In this experiment, the level of cells that shows immune response to gastrin, serotonin, and CGRP is higher in the 2EA and 4EA groups than in the control group. Thus, it is considered that EA stimulation enhances activity of gastrin, serotonin, and CGRP-releasing cells and maintains homeostasis on the digestive enzyme releasing function.

Shapira et al. [45] stated that when EA is applied to a rat that had diabetes, the blood insulin level did not decrease and blood glucose decreased with loss of body weight, which is effective for treatment of diabetes. Chang et al. [46] reported that when stimulating both Zusanli of rats that diabetes is induced by streptozotocin with 15 Hz EA,
blood glucose levels decreased, and susceptibility to insulin increased.

In this experiment, after stimulating Zusanli with EA, immunostaining response of insulin and PP-releasing cell in pancreatic islets is stronger in the 2EA and 4EA groups than the control group. Thus, it is considered that EA stimulation is effective to treat diabetes by enhancing activity of insulin and PP-releasing cells.

After stimulation with EA, the intermediolateral nucleus and lamella VII area marked on the thoracic spinal cord is the area of sympathetic neuron that regulates smooth muscle and sweat glands in the visceral and blood vessel wall, which receive noxious stimulus of visceral or somatic regions [47]. Lamellae I and II areas marked on the lumbar spinal cord are the areas that most often respond to noxious stimulus and temperature stimulus, whereas lamellae III and IV areas play a role in responding to proprioception and light-touch sensation or the functioning related to reflexive movement of voluntary muscle by collateral branches in the brainstem and dorsal horn [47]. It is known that most somatic neurons responding to light stimulus project to lamellae II, III, IV, and V, whereas the neuron responding to noxious stimulus that generally participates in visceral organs and the somatic region projects to lamellae I, V, VII, and VIII of spinal cord. Neurons that control visceral organs and somatic regions in lamellae V, VII, and VIII respond to the stimulus on the muscle below subcutaneous tissue rather than skin stimulus. Thus, if referred pain by visceral organs appears, the type of pain is exhibited as muscle spasm rather than pain on skin [47,48]. Therefore, when treating with acupuncture on acupoints, the needle should be inserted into the deep muscle below subcutaneous tissue if the disease is related to visceral organs, not if the disease is related to pain in musculoskeletal system.

In this experiment, immunohistochemical response to serotonin is shown in the intermediolateral nucleus of 2EA group in the T9-10 area of the spinal cord; a strong immunohistochemical response is shown in some areas of lamellae VII, VIII, and IX. Also, immunohistochemical response to CGRP indicates a strong response in the intermediolateral part of lamellae I, II, and III area of 2EA and 4EA groups in the T9-10 spinal cord area; and a strong response in the intermediolateral part of lamellae I, II, and II of the 2EA and 4EA groups in the L4-5 spinal cord area.

Regarding movements of the GI tract, according to the particularity of organs corresponding to a single acupoint, it is considered that the effect of EA regarding movements of the GI tract is stimulating a nerve through the autonomic nerve center and regulating movements of the GI tract according to the level of various hormones in the blood. Therefore, it is considered that the effect of EA is taken by the collaborative action of nerve stimulation of the autonomic nerve center and hormones secreted in the enteroeendocrine cells of the GI tract.

5. Conclusion

From the results of these experiments, according to the particularity of organs corresponding to a single acupoint, it is considered that the effect of EA regarding movements of the GI tract is stimulating a nerve through the autonomic nerve center and regulating movements of the GI tract according to the level of various hormones in the blood. Therefore, it is considered that the effect of EA is taken by the collaborative action of nerve stimulation of the autonomic nerve center and hormones secreted in the enteroeendocrine cells of the GI tract.

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References


