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Research Article

Effect of acupuncture at three different acupoints on electrical activity of gastric distention-affected neurons in rat medial vestibular nucleus☆,☆☆

Liang Chao[a,b], Wang Yuan[c], Xu Bin[b], Yu Zhi[b,*]

[a] Department of Medicine, Affiliated Hospital to Hebei University of Engineering, Handan 056002, China
[b] Key Laboratory of Integrated Acupuncture and Drugs Affiliated to the Ministry of Education of China, Nanjing University of Chinese Medicine, Nanjing 210046, China
[c] Teaching Management, Shandong Drug And Food Vocational College, Shandong 264210, China

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ABSTRACT

Objective: To observe the effect of gastric distention (GD) and acupuncture at three different acupoints on the spontaneous discharge of neurons in the medial vestibular nucleus (MVN), and to clarify the specific function of the MVN in the central integration mechanism underlying acupuncture regulation.
Methods: GD was conducted using a balloon inserted in the stomach cavity, and acupuncture was performed separately at each of the three acupoints: Zusanli (ST 36), Quchi (LI 11), and Weishu (BL 21). The effect of acupuncture and GD on the spontaneous discharge of MVN neurons was assessed using a glass microelectrode filled with a sodium acetate electrolyte solution containing 1% pontamine sky blue; the discharge signals from the neurons were amplified by the microelectrode amplifier and recorded in the Spike2 system.
Results: GD and acupuncture significantly affected the spontaneous discharge of MVN neurons. Furthermore, acupuncture at Zusanli (ST 36) and Weishu (BL 21) was significantly more effective at altering the discharge of GD-responsive MVN neurons compared with GD-nonresponsive neurons.
Conclusion: GD and acupuncture at three different acupoints affected the electrical activity of MVN neurons. The MVN is involved in the central integration mechanism underlying acupuncture regulation of gastric functions. The effects of acupuncture on gastric function may therefore be mediated via these particular MVN neurons.

1. Introduction

The vestibular system affects balance and proprioception, and plays a key role in the visceral activities associated with the balancing ability. The vestibular receptors send signals to the vestibular nuclei (VN) and other relevant nuclei, and integration between the vestibular system and other systems enables these receptors to regulate body postures and muscular tension to maintain body balance [1,2]. Furthermore, visceral activities such as circulation and digestion are all rapidly and adaptively adjusted [3–5]. Previous studies have found that acupuncture stimulation of specific acupoints can significantly alleviate the symptoms caused by motion sickness (MS), and efficiently relieve nausea, vomiting, and other symptoms [6]. However, the central mechanism and the specific pathways by which acupuncture signals are transferred to the brain are still unclear.

As the VN play important roles in the transfer and processing of vestibular information, we investigated the effect of gastric distention (GD) and acupuncture at three different acupoints on the spontaneous discharge of neurons in the medial vestibular nucleus (MVN). The MVN is the main structure of the VN. We hypothesized

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* Corresponding author.
E-mail address: mickey28282@sina.com (Y. Zhi).

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that the MVN is involved in the central integration mechanism underlying the regulatory effect of acupuncture on gastric function. This study may provide a new direction for research on the gastric regulatory mechanisms of acupuncture.

2. Materials and methods

2.1. Experimental animals

A total of 90 healthy Sprague-Dawley rats of specific pathogen free grade (all 4-month-oldmales weighing 250–300 g) were purchased from the Model Animal Research Center of Nanjing University (certificate of quality No. SCXK 2013-0005). Food and water were available ad libitum, and the rats were housed under controlled environmental conditions. All experimental manipulations were undertaken in accordance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals, published by the National Science Council, China.

2.2. Materials and drugs

Urethane was purchased from Shanghai Qingxi Chemical Technology Co., Ltd., (Shanghai, China). Pontamine sky blue was purchased from Sigma (Chicago, IL, USA). Anhydrous sodium acetate was purchased from Nanjing Chemical Reagent Co., Ltd., (Nanjing, China). Stereotaxic apparatus was purchased from David Kopf Instruments (Tujunga, CA, USA). The microelectrode manipulator was purchased from Narishige Co., (Tokyo, Japan). The extracellular electrophysiological record amplifier was purchased from A-M Systems (Chicago, IL, USA). The Micro 1401-3 biosignal collection and Spike2 analysis system were purchased from CED (London, UK). The H-KWDY-III temperature controller was purchased from Nanjing Quanshui Teaching Equipment Factory (Nanjing, China). The dental drill was purchased from Shanghai Alcott Biological Science Technology Company (Shanghai, China). The WD-1 glass microelectrode controller was purchased from Chengdu Instrument Factory (Chengdu, China). The glass microelectrodes (length 100 mm, outside diameter 1.2 mm) were purchased from Nanjing Quanshui Teaching Equipment Factory (Nanjing, China). Acupuncture needles (0.30 mm × 25 mm) were purchased from Suzhou Medical Supplies Factory (Suzhou, China).

2.3. Recording of neuron discharge

A glass microelectrode (tip 0.5–2 μm) was filled with a 0.5 mol/L sodium acetate electrolyte solution containing 1% pontamine sky blue (impedance 10–20 mΩ). The microelectrode controller was used to localize the microelectrode to the target nuclei, and the discharge signals from MVN neurons were recorded. After preamplification, discharge signals were routed into the biosignal acquisition system. The discharge signals from the neurons were amplified by the microelectrode amplifier and recorded in real time by the Spike2 system. When the neurons emitted spontaneous discharge signals, we adjusted the propeller up and down until the signal-to-noise ratio was stable (the discharge amplitude stabilized at the same level and the signal-to-noise ratio was >1/3), and then stopped the propulsion. The electrode tip was stopped at the discharging neuron, and data were recorded after the discharge stabilized. The signals were recorded at 1 min before intervention as the baseline. After intervention, when the spontaneous discharge stabilized and returned to baseline, the next intervention was started.

2.4. Gastric distention

After 12 h of fasting, the rats were anesthetized by 20% urethane (7 mL/kg). A trachea cannula was used to maintain smooth breathing. The upper abdomen was shaved before the abdominal cavity was cut open, and the liver was moved to expose the pylorus of the stomach and the superior segment of the duodenum. A 2-mm incision was made in the stomach 5–10 mm below the pylorus, and a small balloon made of flexible condom rubber was inserted through this incision into the stomach; the balloon was connected to a sphygmomanometer and a 10-mL syringe. After surgery, the rats were placed in prostrate position. When stable neuron discharges were recorded, the balloon was inflated with air using the syringe until the blood pressure reading reached 30 mm Hg. After 30 s of continuous distention, the balloons were quickly deflated until the blood pressure reading returned to 0 mm Hg. Acupuncture intervention was performed after the neuron discharge background returned to baseline.

2.5. Medial vestibular nucleus localization

Rats were fixed onto the stereotaxic apparatus in prostrate position, and underwent routine craniotomy as follows. In brief, the anterior and posterior fontanelles were exposed and adjusted to the same level. According to the Paxinos and Watson rat brain stereotaxic atlas, the coordinates of the MVN were as follows: −10.56 to −12.3 mm (AP), 0.5–2.0 mm (L), 7.0–8.2 mm (H) [7]. A dental drill was used to perform craniotomy at the appropriate position, and surgical microscissors and microforceps were used to peel off the dura to expose the brain tissue. The surgical positions were then covered by warm liquid paraffin to protect against drying. During the experiments, an electric hot plate was used to maintain the animal's body temperature at 37.0 ± 0.5 °C. After surgery, the animals were allowed to rest for 30 min until all indices were stabilized.

2.6. Group and stimulation

Zusanli (ST 36), Quchi (LI 11), and Weishu (BL 21) [8–10] were used as acupoints on the left side. To eliminate the effect of the order in which the acupoints were investigated, we stimulated the acupoints in a random order. The needle twirling rate was 120–180 twirls/min, and each acupoint was stimulated for 1 min. The next acupoint stimulation was not started until the neuron discharge recovered to baseline.

2.7. Histological localization

After each experiment, a digital display direct current stabilized voltage supply stimulator was used to supply converse direct current (10 μA, 20 min) to the microelectrode, and the pontamine sky blue in the glass microelectrode was sent *via* microelectrophoresis to the electrode tip recording point. The brains were then removed and fixed in 4% paraformaldehyde. After 1 week, 40 to 60-μm-thick brain sections were made using a frozen section instrument, and the location of the microelectrode tip was checked.

2.8. Data collection

The neuron discharge signals were preamplified and sent into a multichannel biosignal collection system for data collection and analysis by the Spike2system. Using discharge frequency (DF, spikes/s) as the observation index, the DF before intervention was used as the baseline (DF_0) and the period of intervention was designated as DF_1. The DF changing rate (R_{DF}) was calculated as $(DF_1-DF_0)/DF_0 \times 100\%$. The absolute value of $R_{DF} \geq 15\%$ was regarded as excitation/inhibition, and $R_{DF} < 15\%$ was regarded as "no change".

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